A Study of Determinants and Prevalence of Rheumatic Heart Disease in Cape Town

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Publications arising from this thesis

- DA Watkins, LJ Zühlke, ME Engel, BM Mayosi. Rheumatic Fever: Neglected Again. Science 2009; 324:37 (Letter)

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- Zühlke L, Engel ME. Rheumatic Heart Disease: The time has come. Clinical Epidemiology 2011; 3: 171. (Letter)

- Engel, ME; Stander, R; Vogel, J; Adeyemo, AA; Mayosi, BM. Genetic susceptibility to acute rheumatic fever: a systematic review and meta-analysis of twin studies. PLoS One. 2011;6(9):e25326.

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Abbreviations

A.S.A.P.	Awareness, Surveillance, Advocacy, Prevention Programme in ARF/RHD
ARF	Acute rheumatic fever
β	beta
βHS	beta-Haemolytic streptococcus
CDC	Centres for Disease Control and Prevention
CI	Confidence interval
CPRs	Clinical prediction rules
CRF	Case report form
DZ	Dizygotic
GAS	Group A *streptococcus*
MZ	Monozygotic
OR	Odds ratio
PPS	Probability proportional to size
RHD	Rheumatic heart disease
ROC	Receiver operating curve
SA	South Africa
WHF	World Heart Federation
WHO	World Health Organisation

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Abstract

BACKGROUND

Rheumatic Heart Disease (RHD) is a post-infectious immune disease ascribed to an interaction between a rheumatogenic strain of group A *streptococcus*, a susceptible host who lives in poor social conditions with limited access to medical facilities. The disease process begins with repeated group A streptococcal (GAS) infections, which, subsequently result in acute rheumatic fever (ARF). In the absence of intervention, repeated bouts of ARF in turn, may result in progression to RHD, particularly in those ARF patients with cardiac involvement. The prevalence of ARF and RHD in developed countries has shown considerable decline during the last century, largely attributed to improved living conditions and access to healthcare. Epidemiological data from developing countries, while scant, indicate a continued high prevalence of GAS-positive pharyngitis and RHD. Furthermore, while much is known about the social factors and the microbial agent that predispose individuals to ARF, little progress has been made in elucidating genetic susceptibility factors that are reproducible in different populations.

AIMS OF THIS THESIS

This thesis aimed to establish determinants of RHD as well as to document the prevalence of RHD in South African school children. The specific objectives of each study component were as follows:

- To derive quantitative estimates of the size of the genetic contribution to the risk of developing ARF/RHD.

- To determine the prevalence of GAS carriage and *emm* strains of GAS isolates among asymptomatic children enrolled in primary and secondary school.
- To describe the epidemiology of GAS among 3- to 15-year old children with pharyngitis attending primary health care facilities.
- To develop a clinical prediction rule for diagnosis of GAS throat infection that is valid for children aged 5-15 years in the primary care setting within the South African context.
- To determine the prevalence of echocardiographically-proven rheumatic heart disease in school children.

METHODS

The systematic review method was used to investigate population-based twin studies on acute rheumatic fever and rheumatic heart disease for evidence for a genetic effect in disease susceptibility and/or progression (**Study 1**). Langa and Bonteheuwel, two juxtaposed township communities of Cape Town with significant socioeconomic differences was the setting for all the primary studies. Healthy children in primary and secondary schools were screened for GAS carriage by throat swab culture; GAS isolates were subjected to *emm* typing to provide insight into vaccine development (**Study 2).** The prevalence of RHD in healthy learners was established by echocardiography using specifically designed criteria (**Study 5**). For the clinic-based study, children aged 5-15 years with pharyngitis rendered throat swabs for microbiological culture (**Study 3**) while for developing the clinical prediction rule (CPR) (**Study** 4), a multivariate logistic regression model was constructed incorporating variables with evidence of association with throat swab culture result on univariate analysis.

RESULTS AND DISCUSSION

Meta-analysis of pooled data from 435 twin pairs from six studies revealed a strong concordance risk for ARF (OR 6.39; 95% CI, 3.39 to 12.06; $p < 0.001$) with an estimated heritability across all the studies of 60%. The populations-based studies among healthy learners revealed a carriage estimate for GAS of 3.2% (95% CI: 2.13 – 4.39 %) with < 50% of *emm* types in our carrier strains represented in the latest proposed 30-valent vaccine. The prevalence of echocardiographically-confirmed RHD was 28.7 per 1,000 (95% CI, 22.7 – 35.7 per 1,000). A strong socioeconomic gradient was evident with participants from the poorer community having higher rates of carriage and asymptomatic RHD. The period prevalence of GAS pharyngitis in children attending primary heath care clinics was 21.6% while the incidence of pharyngitis and GAS pharyngitis was 0.837/100 person-years and 0.180/100 person-years respectively. Existing CPRs were found to have low applicability in our setting and subsequently, a South African CPR was developed.

CONCLUSION

The novel findings of this thesis provide evidence for genetic susceptibility for RHD, justifying genome wide association studies in RHD patients in the future. This research also emphasises the need for primordial prevention measures to reduce the prevalence of GAS carriage and asymptomatic RHD. Next, it presents the latest report of a rather unexpectedly low incidence of GAS pharyngitis in children from poorer communities. Finally, this thesis provides an attempt at clinical prediction of GAS pharyngitis in the absence of laboratory confirmation of GAS infection. Once confirmed in other populations, the findings of this thesis will make a sizeable impact on reducing the prevalence of RHD in our setting.

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1 BACKGROUND TO THE THESIS

1.1 PATHOGENESIS AND COURSE OF RHEUMATIC HEART DISEASE

Rheumatic Heart Disease (RHD) is a post-infectious immune disease that is associated with significant morbidity and mortality in children and young adults living in developing countries. Acute rheumatic fever (ARF), the precursor to RHD is a multifactorial disorder that is caused by an interaction between a rheumatogenic strain of group A β-haemolytic *Streptococcus* and a susceptible host who lives in poor social (Azevedo et al., 2012). Initially described as long as four centuries ago (Stollerman, 2001), and despite many advances of the twentieth century, RHD remains a fascinating yet challenging condition as regards the precise processes involved in the development of the disease.

The pathogenesis of RHD is characterised by three stages namely, Group A streptococcal (GAS) infection, ARF and RHD (Figure 1.1). The disease process begins with a GAS infection in a susceptible individual. Repeated GAS episodes subsequently result in the development of ARF in 0.3 - 3% of individuals which may or may not include cardiac involvement (Stollerman, 1995). Without intervention, cumulative attacks of ARF may, in due course, result in progression to established RHD, particularly in those ARF patients with cardiac involvement. In this chapter, I discuss the determinants (i.e. agent and host), epidemiology and prevention of GAS pharyngitis, ARF and RHD.

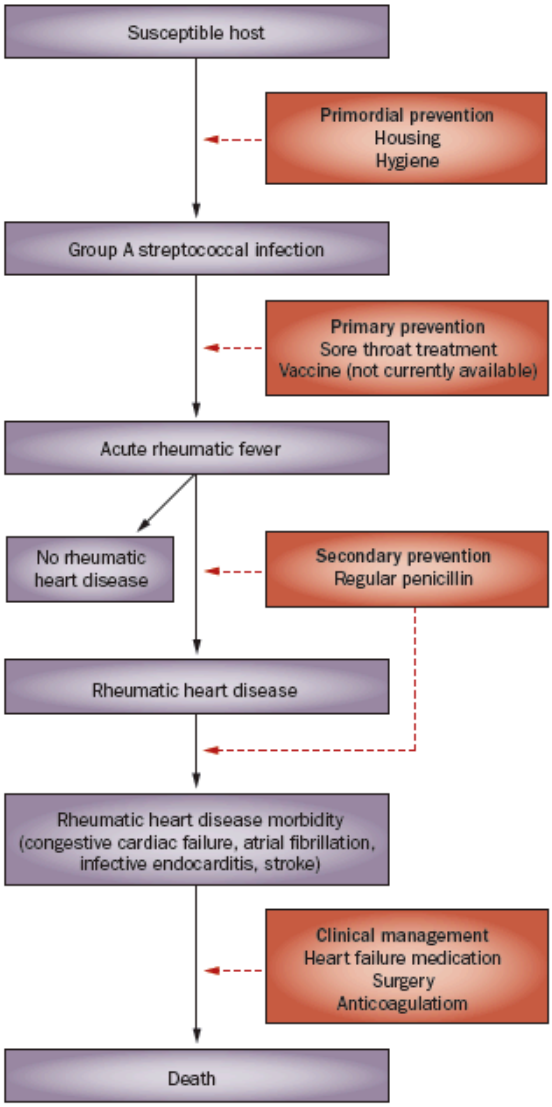


Figure 1.1. Progression from GAS infection to established RHD – (Steer and Carapetis, 2009)

1.2 THE AGENT: INFECTION AND IDENTIFICATION OF GAS

Infection with Group A β-haemolytic *Streptococcus* (Figure 1.2), a gram-positive bacterium also known as *Streptococcus pyogenes,* results in a variety of human such as pyoderma and pharyngitis, and invasive group A streptococcal diseases (Box 1.1). Following the initial

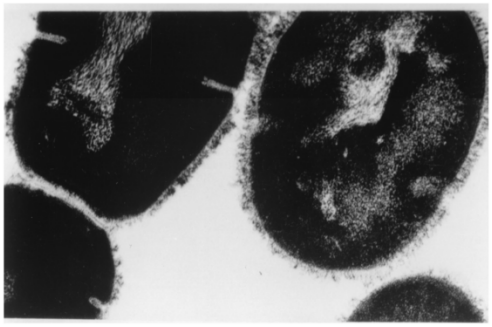


Figure 1.2. Electron micrograph of group A M type 24 streptococci. Clearly visible are the surface fimbriae or hairlike projections indicating the presence of M protein on the surface of the streptococci (Cunningham, 2000).

infection, repeated GAS pharyngeal and skin infections, despite being classified as superficial, might give rise to more serious GAS-related illnesses such as acute post-streptococcal glomerulonephritis, ARF and RHD (Cunningham, 2000, Carapetis et al., 2005b, Stollerman, 1975, Marijon et al., 2012). Haemolytic streptococcal species, so-called due to the pattern of haemolysis (due to haemolysin streptolysin S) produced round colonies on blood agar plates following incubation, were suspected as the causative agent of ARF as long ago as at the turn of the previous century. Subsequent clinical, bacteriological, and epidemiological contributions by a number of investigators, including a system of serological classification by Rebecca Lancefield, confirmed the causative role of

Streptococcus pyogenes, classified as GAS with the Lancefield serogrouping system, in the development of ARF (Stollerman, 1975).

Box 1.1 Group A streptococcal-related diseases (Steer et al., 2007, Cunningham, 2000, Marijon et al., 2012).

Pharyngitis and Scarlet Fever

Pyoderma and Streptococcal Skin Infections

Invasive Streptococcal Disease:
- Streptococcal Toxic Shock Syndrome
- Necrotizing Fasciitis
- Septicaemia

Post-Infectious Sequelae
- Rheumatic fever
- Acute post-streptococcal glomerulonephritis
- Reactive arthritis
- PANDAS†

†PANDAS, Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections

1.2.1 Laboratory Aspects of GAS

In culture, GAS can be differentiated from normal throat flora by their distinct appearance as β-haemolytic colonies on 5% sheep agar incubated under anaerobic conditions (Figure 1.3). Differences in the cell-wall polysaccharide or lipoteichoic acid components of streptococci allow for the further differentiation of β–haemolytic streptococci (βHS) into serotypes using the Lancefield grouping system; for example, GAS is distinguished by its

group A carbohydrate, composed of N-acetylglucosamine linked to a rhamnose polymer backbone (Cunningham, 2000, Lancefield, 1941).

Figure 1.3. Pattern of haemolysis around streptococcal colonies distinguishing beta (1) with large zones of complete haemolysis around colonies from alpha (2) with its incomplete haemolysis and dark green agar under the colonies. Photo: M Engel, 2012

Other βHS serotypes include Groups B, C, F, and G. Grouping antisera employing slide agglutination techniques, allow for accurate determination of subgroups following culture (Facklam, 1997). In response to the time constraints of microbiological culture, which requires a number of days within which to isolate the bacteria, and the urgency to commence treatment, a number of rapid GAS identification tests have been developed over the years to assist the clinician in achieving a diagnosis at the point of care (Gerber and Shulman, 2004, Maltezou et al., 2008).

1.2.2 Molecular Aspects of GAS

Earlier work by Rebecca Lancefield established a type-specific surface antigen on GAS, the M-protein, that enabled further classification into antigenically distinct pathogenic strains (Lancefield, 1941). The M-protein, consisting of two polypeptide chains extending from the cell wall, comprises four blocks of amino acid residues (Fischetti, 1989) (Figure 1.4).

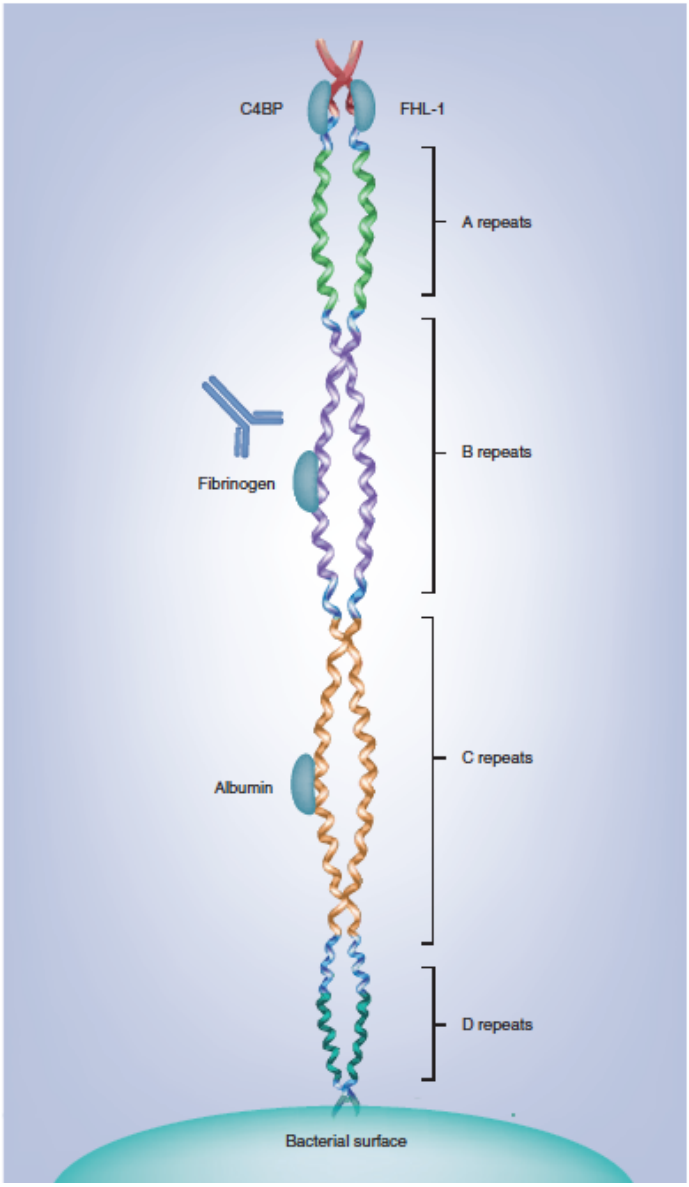


Figure 1.4. Schematic illustration of M protein. Individual M proteins form coiled coils, which extend from the bacterial surface. Two coils wind around each other, forming a dimer that is stabilized by intermolecular interactions between amino acids in both strands. The hypervariable N-terminal region, lacking helical structure, is followed by the A, B, C and D repeat regions (Smeesters et al., 2009).

The variability within the N-terminal region allows for differentiating GAS strains (i.e. M-typing) using specific M-typing antisera (Cunningham, 2000). The M-protein is encoded by the *emm* gene and is the major target for the immune system (Scott et al., 1985). Recent advances in molecular biology have resulted in DNA sequence-based methods (emm-typing) making it possible to differentiate the more than 200 variations in the *emm* sequence (Beall et al., 2000, CDC Protocol for emm typing available at http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm). The N-terminal region of the M-protein is the target for vaccine development, having been shown to evoke antibodies with the greatest bactericidal activity, while showing no cross-reaction with human tissues (Dale, 2008). Amino acid sequences of M Proteins of 30 *emm types* have been included in the latest vaccine initiative and preliminary results indicate bactericidal killing of >40 % in over 90% of the vaccine serotypes and additional cross-opsonic activity against 60% of 40 non-vaccine serotypes of GAS evaluated (Figure 1.5) (Dale et al., 2011).

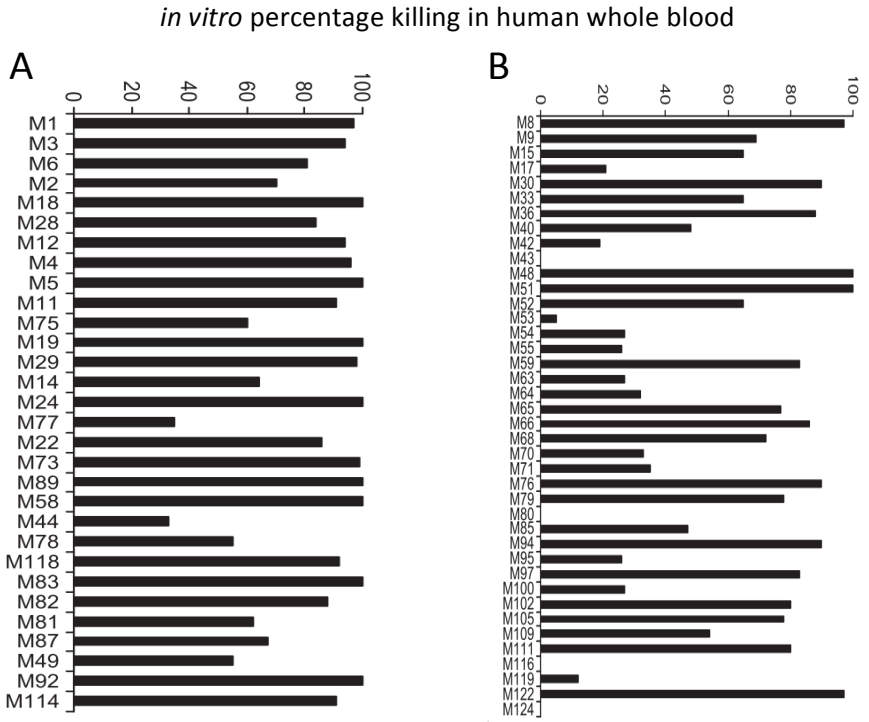


Figure 1.5. Percentage killing by bactericidal antibodies evoked by the 30-valent vaccine. **A.** Vaccine serotypes**. B**. Non-vaccine serotypes (Dale et al., 2011).

1.3 THE HOST: IMMUNE RESPONSE AND PREDISPOSING FACTORS

The development of RHD is ascribed to an interaction between a rheumatogenic strain of group A *streptococcus*, a susceptible host and environmental factors (Figure 1.6) (Carapetis et al., 2005a). In this section, I review the immunological sequence of events occurring in the host following an initial pharyngeal GAS infection, together with host susceptibility and environmental factors.

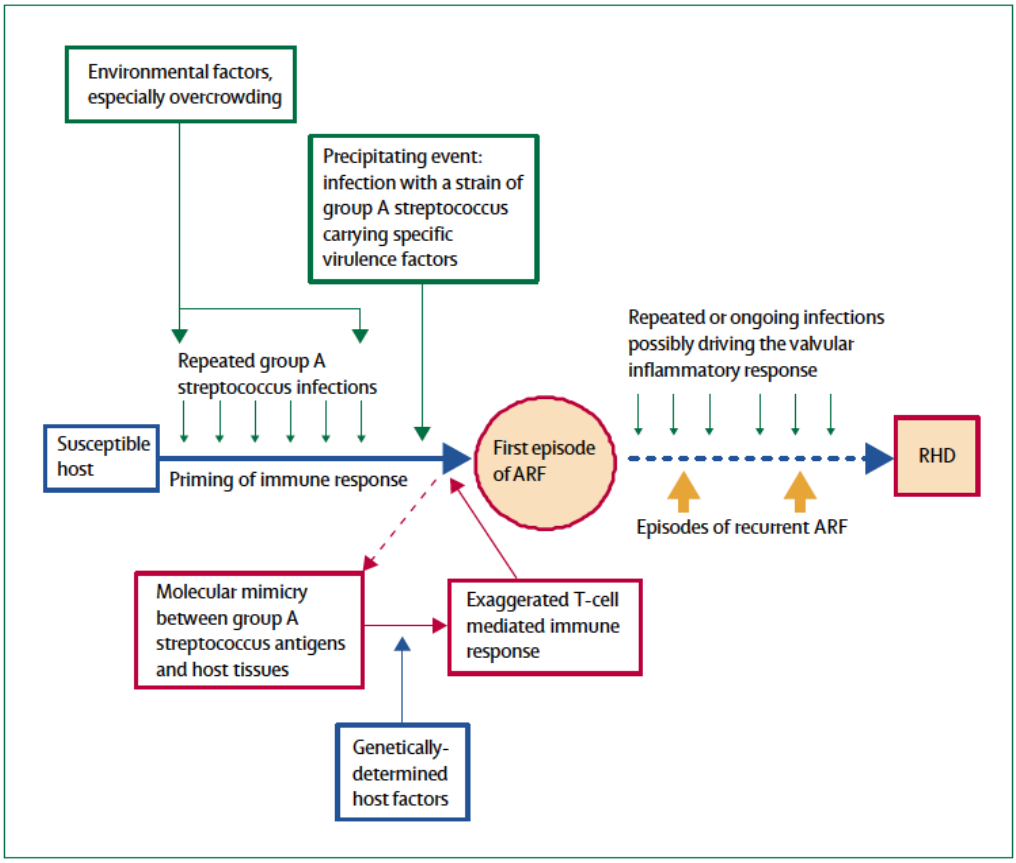


Figure 1.6. Pathogenic pathway for ARF and RHD (Carapetis et al., 2005a).

1.3.1 Host Response Mechanisms

Figure 1.7 illustrates the sequence of immunological events leading to the development of RHD (Guilherme et al., 2007). The development of ARF and RHD following an episode of GAS pharyngitis is brought about through humoral and cellular mediated molecular mimicry whereby some degree of homology between the GAS M protein and host structures, result in the recognition, and subsequent damage of host tissues by B- and T-lymphocytes (Guilherme et al., 2011). Several cross-reactive epitopes between human proteins and the M protein B block have been documented including laminin in heart valvular tissue, cardiac myosin and vimentim (Cunningham, 2000, Guilherme et al., 2005). Binding to the endothelial surface of the valve by cross-reactive antibodies leads to inflammation, cellular infiltration (supported by evidence for upregulation of the vascular cell adhesion molecule-1 (VCAM-1) ((Roberts et al., 2001) and valve scarring (Galvin et al., 2000). Further support for the involvement of the humoral immune system is seen in the recovery of anti-GAS antibodies from the sera of ARF patients (Carapetis et al., 1996).

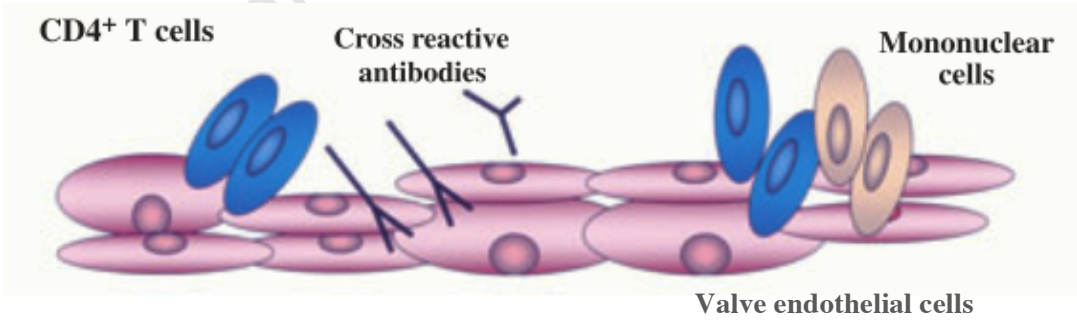


Figure 1.7. Major events triggering rheumatic valvular lesions in RHD. Heart tissue cross-reactive antibodies bind to the surface of the valve endothelial and facilitate mainly CD4+ T-cell infiltration. Streptococcal primed T cells trigger an autoimmune reaction resulting in the production of inflammatory cytokines by mononuclear cells. Regulatory IL4+ cells are found in the valvular tissue leading to the progression of valvular lesions. (Guilherme et al., 2007)

1.3.2 Genetic Predisposition in the Host

Various studies suggest that host factors may play a role in individual susceptibility to ARF. As early as in the late 1800s, Walter Cheadle noticed that ARF appeared to occur in clusters in families (Stollerman, 1975). Others have also since found a frequent familial prevalence of ARF as compared with matched control families (Honeyman and Davis, 1971, Wilson and Schweitzer, 1937) leading some to conclude the possibility of simple recessive mendelian inheritance (Honeyman and Davis, 1971). A five-year study of rheumatic and non-rheumatic families confirmed a high prevalence of ARF among individuals from rheumatic families who contracted ARF or developed RHD (Quinn and Federspiel, 1967). Furthermore, familial predisposition has also been demonstrated in an evaluation of 19 multiplex families segregating for ARF (Hafez et al., 1985).

Associations of ARF/RHD with various genes have been investigated. The initial reports of HLA polymorphisms provided evidence of protective HLA class II gene alleles (e.g., DRB1*13, DQA1*0103) and susceptibility alleles (e.g. DRB1*0701, DQA1*0201) to the risk of developing RHD (Guedez et al., 1999, Kudat et al., 2006). A meta-analysis of 10 studies investigating disease risk associated with HLA-DR antigen expression supported an association between major histocompatibility complex class II alleles and risk for RHD. (Carlquist et al., 1995). Increased frequencies of specific HLA antigens in patients with ARF/RHD have been reported (Visentainer et al., 2000, Wani, 1997, Hernandez-Pacheco et al., 2003a). There are also reports of non-HLA genes that may be associated with ARF including Toll-Like Receptor-2 (Berdeli et al., 2005), Fc fragments of immunoglobulin G IIA and IIIB (Berdeli et al., 2004), Tumour Necrosis Factor-α (TNF-α) (Sallakci et al.,

2005); and with RHD TNF-α (Hernandez-Pacheco et al., 2003b), Transforming growth factor-beta (Hernandez-Pacheco et al., 2003b, Chou et al., 2004a), mannose-binding lectin (Jin et al., 2001) and angiotensin I-converting enzyme (Chou et al., 2004b, Davutoglu and Nacak, 2005). Recently, Guilherme and colleagues reviewed the gene polymorphisms associated with rheumatic fever in detail (Box 1.2) (Azevedo et al., 2012).

The three study designs used for testing the level of genetic involvement in a disease include familial aggregation, twin, and adoption studies. The familial aggregation study assesses the familial recurrence of a condition, enabling for the evaluation of the prevalence of a disease within families (Khoury, 1998); diseases having a genetic predisposition will have a higher rate of prevalence compared to the general population. Thus, where relatives are affected with the disease, other family members are expected to have an increased risk of developing that disease. In familial aggregation studies investigating ARF/RHD, non-diseased relatives of both probands and matched controls (according to age and gender), are observed for the presence or absence of ARF/RHD or rheumatic manifestations defined by the proband. Comparisons according to the odds of exposure (i.e. positive family history) in the case subjects are compared to the odds of exposure in the control subjects. By using a two-by-two table; measures of association such as odds ratio could further be derived.

Box 1.2. Gene polymorphisms associated with RF and/or its manifestations (Azevedo et al., 2012)

Gene polymorphisms	Assoc.	OR	*P*
Innate immunity			
MBL AA	RHD	1.99	≤0.02
MBL YA/YA & YA/XA	RHD	2.48 & 2.42	0.035 & 0.001
MBL defectives alleles	AoR	3.5	0.0022
TLR2 Arg753Gln & Arg753Arg	RF	97.1 & 0.01	$<10^{-3}$ & $<10^{-3}$
FCN2 -986/-602/-4 G/G/A & A/G/A	RHD	1.6 & 0.3125	0.021 & 0.008
Adaptive immunity			
IL1RN A1 & A1A1	SRHD	0.11 & 0.092	0.031 & 0.017
IL1RN A1A1	RHD	2.2	<0.05
IL-10 -1082 AA & GG	RHD/ MVL	3.1 & 5.2/5.2 & NS	<0.05 & <0.05/<0.05 & NS
FCγ RIIA RR & RIIIB NA2	RF	4.98 & NS	0.0022 & NS
TNFα G-308A & G-238A	RF	1.4 & 1.9	0.026 & 0.015
TNFα G-308A	RF/RHD	3.4/3.3	<0.0032/<0.0055
TNFα G-308A & G-238G	RHD/ MVL	10.8 & 14.1/8.65 & NS	$<10^{-3}$ & $<10^{-3}$/$<10^{-3}$ & NS
TNFα -308A & -238A	OCD	NC & NC	<0.0005 & 0.0099
TNFα -308AA	RHD/ MVL	5.7/10.6	$<10^{-3}$/<0.05
TGF-β1 C-509T & T869C	RHD	1.49 & NC	$<10^{-3}$ & 0.04
TGF-β1 C-509T & T869T & T869TT	RHD	1.78 & 1.89 & 3.37	0.04 & 0.02 & 0.02
CTLA-4 +49GG	RHD	3.1	0.016
Others			
ACE II	RHD	NC	<0.003
ACE II	RHD	2.12	0.02

MBL, mannose-binding lectin; TLR2, toll-like receptor-2; FCN2, ficolin-2; TNFa, tumor necrosis factor-a; TGFb, transforming growth factor-b; CTLA-4, cytotoxic T-lymphocyte antigen-4; IL1RN, interleukin-1receptor antagonist gene; IL10, interleukin-10; ACE, angiotensin-converting enzyme; AoR, aortic regurgitation; MVL, multivalvular lesion; OCD, obsessive compulsive disorder; NS not significant, NC not calculated.

Twin and adoption studies provide the opportunity to delineate the contribution of genetics versus environment to the development of diseases. A comparison of the concordance rates between monozygotic (MZ) twins, who are genetically identical, and dizygotic twins who, on average, share 50% of their DNA, serves to indicate the role of genetics in the aetiology of a disease (Farrer LA, 1998). Studies of twins have to date, provided compelling evidence of a genetic component in a number of diseases including psychosis (Allan et al., 2009), stroke (Flossmann et al., 2004), breast cancer (Mack et al., 2002), osteoporosis (Miakotkin and Benevolenskaia, 2008) and familial Mediterranean fever (Shohat et al., 1992).

1.3.3 Role of Environment

During the last century, the prevalence of ARF and RHD in developing countries has not shown the decline observed in industrialised countries, which has largely been attributed to poor living conditions and reduced access to healthcare (Quinn, 1989). A recent review presented evidence for the role played by socioeconomic factors such as overcrowding, nutrition and access to medical services in susceptibility to developing ARF (Steer et al., 2002). As can be expected, lower socioeconomic conditions predispose to a higher burden of GAS pharyngitis (Nandi et al., 2001).

Contrasting views of the role of environment have also been presented. In the earlier part of the previous century, Wilson reported no increased risk of ARF in rheumatic siblings living in poorer conditions as compared with those from wealthier environments (Wilson and Schweitzer, 1937). Thus, environmental factors may only partially explain susceptibility to developing ARF (Azevedo et al., 2012).

1.4 THE DISEASE: GAS PHARYNGITIS, ARF AND RHD

1.4.1 GAS Pharyngitis

Pharyngitis, from Greek, '*pharynx*', meaning throat and '*-itis*', suffix meaning inflammation, refers to swelling accompanied by pain or discomfort in the throat, often resulting in difficulty with swallowing. Most cases of sore throat are due to viral infections such as influenza, requiring symptomatic treatment before eventually self-resolving. GAS pharyngitis specifically refers to sore throat resulting from an infection with GAS. Surface streptococcal ligands on the bacterium bind to specific receptors on host pharyngeal cells thereby inducing ingestion (LaPenta et al., 1994) (Figure 1.8). Failure to eradicate streptococci from the pharynx occurs in about 1/3 of non-treated cases, giving rise to the carrier status in those individuals harbouring intracellular GAS (Markowitz et al., 1993).

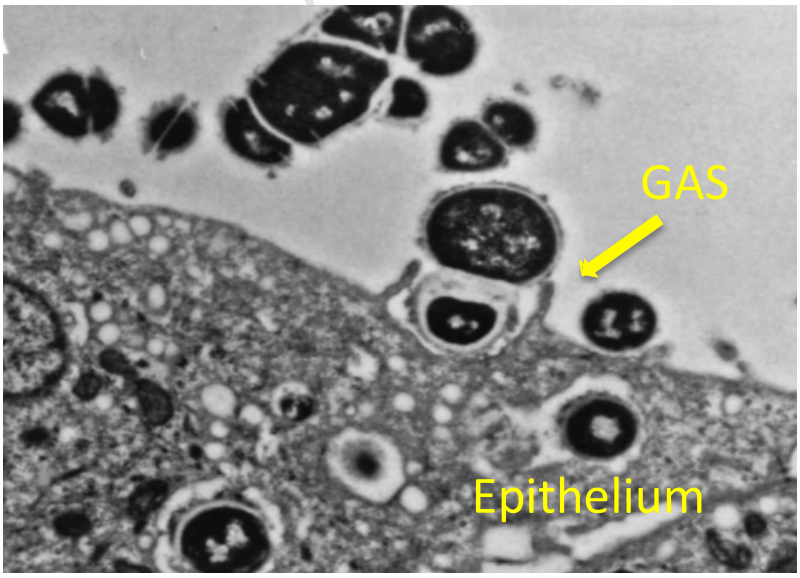


Figure 1.8. Electron micrograph (x12,700 magnification) demonstrating the attachment and internalization of streptococci by human cultured pharyngeal cells (Cunningham, 2000).

1.4.1.1 Symptoms and Diagnosis of GAS Pharyngitis

The management of pharyngitis is of major public health concern and the appropriate use of antimicrobial therapy is an important strategy in the reduction of the incidence of ARF and RHD (Karthikeyan and Mayosi, 2009, Robertson et al., 2005a). GAS pharyngitis is characterised by throat pain with none or some of the symptoms of fever, chills, malaise, headache, abdominal pain, nausea, and vomiting (Wannamaker, 1972) (Box 1.3) (World

Box 1.3 Clinical signs and symptoms of group A streptococcal upper respiratory tract infection, by patient age group (World Health Organization., 2004) .

Clinical signs and/or symptoms	Infants	School-age children	Adolescents and adults
Anterior cervical lymphadenitis (tender nodes)	++++[b]	++++	++++
Close contact with an infected person	++++	++++	++++
Scarlatiniform rash	Unusual	++++	++++
Excoriated nares	++++	Unusual	Unusual
Tonsillar or pharyngeal exudate	Uncommon in infants younger than three years of age	++++	++++
Positive throat culture	++++	++++	++++
Fever	++ (Not specific)	++ (Not specific)	++ (Not specific)
Acute onset of symptoms	+ (Unusual)	++ (Not specific)	++ (Not specific)
Abdominal pain	++	++	+ (Unusual)
Coryza	++	Unusual	Unusual
Erythema of the pharynx	Not specific	Not specific	Not specific
Hoarseness	Unusual	Unusual	Unusual
Cough	Unusual	Unusual	Unusual

[a] Modified from (*11*).
[b] The symptoms are classified semiquantitatively as being: less typical (+); more typical and frequent/moderately suggestive (++); and almost always present in patients with streptococcal pharyngitis/very suggestive (++++).

Health Organization., 2004) . The number of national and local guidelines that have been developed over the years bears testimony to the importance of correctly identifying GAS pharyngitis to ensure timely treatment (Bisno et al., 1997, Wachtler and Chenot, 2011, Matthys et al., 2007, Marres, 2008, Kerdemelidis et al., 2009, Chiappini et al., 2012). While a number of rapid diagnostic tests has been developed in recent years, microbiological culture of throat swabs remains the gold standard for diagnosing the presence of GAS upon presentation of pharyngitis.

1.4.1.2 Clinical Prediction Model Development

Clinical prediction rules (CPRs) serve to aid diagnostic evaluation, particularly in low-resourced settings where laboratory facilities may not be available. They are practical decision tools, incorporating a limited number of variables obtained at the time of examination, to derive a probability score for directing a course of action (Wasson et al., 1985, Laupacis et al., 1997).

Development of Clinical Prediction Rules

The development of CPRs involves a preceding decision phase that will impact on the final model's application to the disease of interest (Box 1.4). Selecting candidate variables generally follows a systematic review of the literature, taking into account pre-existing knowledge of clinical predictors of the disease of interest (Spiegelhalter, 1986). It is often preferable to limit the number of variables, especially given that simpler models are easier to apply in practice (Laupacis et al., 1997). Following enrolment and collection of the patient data as per the chosen variables, it is essential that data are evaluated in terms of

quality, especially documenting the reasons for missing values and possibly excluding those predictors for which the cut-off threshold (usually < 5%) for missing data has been exceeded.

Box 1.4 Decisions affecting CPR model development (Royston et al., 2009).

1. Selecting clinically relevant candidate predictors for possible inclusion in the model
2. Evaluating the quality of the data and judging what to do about missing values
3. Data handling decisions such as combining or creating new variables
4. Choosing a strategy for selecting the important variables in the final model
5. Deciding how to model continuous variables
6. Selecting measure(s) of model performance or predictive accuracy.

Statistical Procedures in Developing a CPR

A univariate analysis is conducted as the preliminary step to evaluate the potential contribution of the individual variables, based on their respective significance levels of association with the outcome of interest, to the final model. Regardless of the univariate analysis results though, variables known to be associated with the disease of interest should be included in developing the final model. The backward elimination technique is commonly used to derive the final model (Steyerberg et al., 2000); in brief, a series of logistic regression procedures are conducted, initially incorporating all the candidate variables with the systematic removal of those variables failing to achieve a predetermined

significance level after each round of testing. Overall performance of the prediction model is assessed using the receiver operating curve (ROC) where sensitivity is plotted against the false positive rate for different cut-off points of a parameter and the area under the curve is a measure of the distinguishing ability of the model, also known as the c-statistic (Zweig and Campbell, 1993). Values closer to 1 indicate relatively superior performance as compared with values of 0,5. Following the derivation of the best-fit model, beta coefficients are generated using the '*logit*' (in Stata®) command and transformed by multiplying by a factor, into points, which constitute the scoring chart. The final step is the validation of the prognostic model, where the model's performance is evaluated in different groups of patients (Altman et al., 2009).

1.4.1.3 Clinical Prediction Rules for GAS Pharyngitis

In resource-limited settings, sending throat swabs for culture is not always feasible given the cost of the investigations and the waste incurred if patients do not return for follow up of results. In addition to the guidelines provided by the World Health Organisation (WHO) (WHO, 1995) on empiric treatment with antibiotics in the absence of laboratory confirmation, various CPRs to predict streptococcal sore throat have been derived over the last decade, many of which were from developed country settings (Wald et al., 1998, McIsaac et al., 1998, Breese, 1977, Centor, 2009, Attia et al., 2001, Joachim et al., 2010). Table 1.1 contains some examples of well-known CPRs for GAS pharyngitis together with scores given to the presence or absence of specific variables.

CPRs for the prediction of GAS-positive pharyngitis in children aged 3 to 18 years presenting with sore throat have been subjected to systematic review recently (Shaikh et al.,

2011). Thirty-four studies (totaling 24,418 patients) from clinics and emergency departments from a number of resource settings were included. Five of the current prediction rules were inaccurate in providing a definitive diagnosis of streptococcal pharyngitis leading the authors to support rapid testing or throat culture in suspected GAS cases as suggested by the American Academy of Pediatrics, the American Heart Association, and the Infectious Disease Society of America.

It is imperative to test and validate CPRs in local settings in different groups of patients before they are rolled out as standard practice of care (McGinn et al., 2000). In fact, two of the rules included in the table above (Smeesters et al., 2006, Steinhoff et al., 2005) were developed in resource-constrained scenarios in response to poor performance of CPRs from developed country settings. The 3-variable Steinhoff rule, which presents a modified version of the Abu Reesh rule was developed in the Abu Reesh Hospital in Cairo following the enrolment of 410 participants aged 2 to 12 years who presented at the children's hospital with pharyngitis, while the CPR developed by Smeesters prospectively included 220 patients from three public hospitals of Brazil over a nine month period (Smeesters et al., 2006).

Table 1.1 Summary of clinical prediction rules (modified from (Fischer Walker et al., 2006)).

Rule	Clinical signs/symptoms	Score if present	Score if absent
Abu Reesh	Exudate or enlarged lymph nodes	1	0
Attia et al	Moderate to severe tonsillar swelling	1	
	Moderate to severe large cervical nodes	1	
	Scarlatiniform rash	2	
	Absence of moderate to severe coryza	1	
Breese et al	Season	1-4	
	Age	1-4	
	White blood cell count	1-6	
	Fever (>100.5°F)	4	2
	Sore throat	4	2
	Headache	4	2
	Abnormal pharynx	4	2
	Abnormal cervical lymph nodes	4	2
	Cough	2	4
Centor et al	Tonsillar exudate	1	0
	Swollen tender anterior cervical lymph nodes	1	0
	Cough	0	1
	History of fever	1	0
McIsaac et al	Temperature >38°C	1	0
	Cough	0	1
	Tender anterior cervical lymph nodes	1	0
	Tonsillar swelling or exudate	1	0
	Age in years 3–14 / 15–44 / ≥ 45	1 / 0 / -1	0
Smeesters er al	Age ≤35 /36-59/≥60 months	20 / 6 / 2	
	Viral signs: 1/≥ 2	7 / 10	
	Bacterial signs: 1 / ≥ 2	-2 / -4	10
Steinhoff et al (3-variable)	Enlarged lymph nodes	1	0
	Rash	0	1
	Rhinitis	0	1
Wald et al	Age (5–15 years)	1	0
	Season (November–May)	1	0
	Fever (>38.3°C)	1	0
	Enlarged or tender lymph nodes	1	0
	Erythema, swelling, or exudate on pharynx or tonsils	1	0
	Upper respiratory tract symptoms	0	1
WHO	Exudate and large, tender lymph nodes	1	0

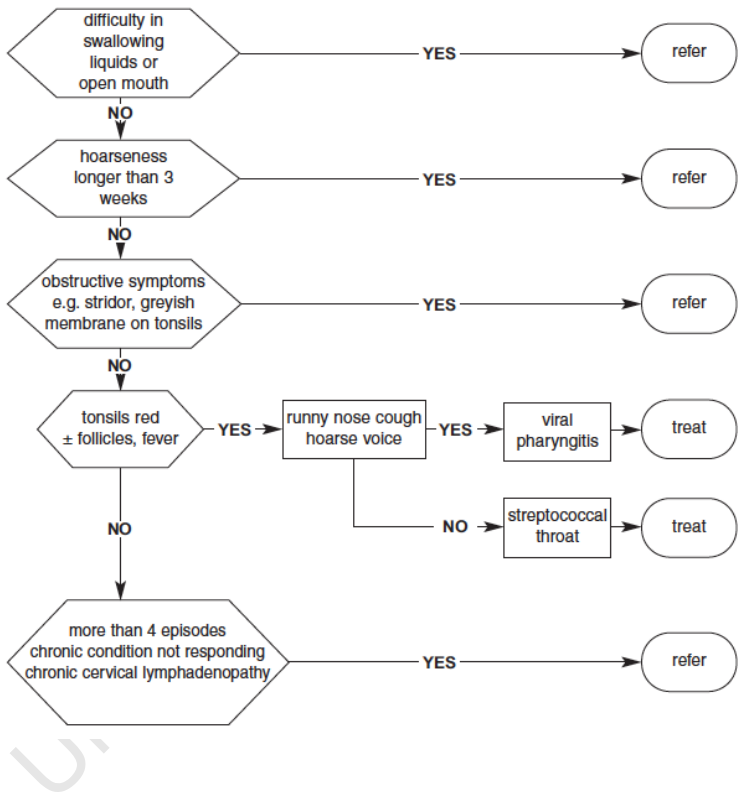
1.4.1.4 Treatment of GAS pharyngitis in SA

In South Africa, guidelines for the management of pharyngitis (including bacterial tonsillitis) are provided in the Department of Health publications, *Standard Treatment Guidelines and Essential Drugs List (EDL) for South Africa, Primary Health Care 2003 Edition (Department of Health, 2006)* and *National Guidelines on the Primary Prevention and Prophylaxis of Rheumatic Fever and Rheumatic Heart Disease for Health Professionals at Primary Level (Department of Health, 1999)* . Clinical features suggestive of β-haemolytic streptococci group A are sore throat, inflamed tonsils with exudate, tender and enlarged cervical lymph nodes and often, sudden onset of fever as illustrated in Box 1.5.

The widespread, often inappropriate use of macrolide antibiotics, which are often used in the treatment of sore throat as an alternative to penicillin, is adding to the growing problem of bacterial resistance to antimicrobials (Silva-Costa et al., 2012). A recent study of prescribing in the South African private sector revealed that over 70% of consultations resulted in the prescription of an antimicrobial agent and that antimicrobials were prescribed, even when the cause of the illness was stated to be viral (Katende-Kyenda et al., 2006). Elsewhere, healthy volunteers given azithromycin and clarithromycin showed a significant increase in the proportion of resistant streptococci in the pharynx within a few days, which remained high up to 180 days later (Malhotra-Kumar et al., 2007). In many parts of the world, antimicrobial resistance to macrolides is increasing, an example being Greece where a prevalence of resistant GAS has been reported to be over 30% prevalence of (Syrogiannopoulos et al., 2004). By comparison, only 60,6% of respiratory isolates of *Streptococcus* pneumonia collected from January to December 2010 from various

geographical regions of South Africa were susceptible to erythromycin (Zietsman and A.J., 2011).

Box 1.5 South African EDL: Diagnosis and Treatment of streptococcal infection in children aged 3 - 15 years. (Department of Health, 2006)



Viruses are the most common cause of acute pharyngitis and a cross-sectional study performed in South Africa in 1979 found that only 33.2% of patients who presented with sore throat had GAS on culture of throat swabs (van Zyl et al., 1981). Therefore, given that antimicrobial therapy is not effective or warranted in at least two-thirds of cases of

pharyngitis, it would thus be ideal to correctly identify those patients with streptococcal pharyngitis who would benefit from antimicrobial therapy while avoiding antimicrobial use in pharyngitis. There is no CPR for application in the South African population and thus, the diagnosis of streptococcal pharyngitis is made on suggestive clinical features and empirical antimicrobial therapy is prescribed (Brink et al., 2004). This practice is endorsed in local guidelines, at least for the age group at high risk for ARF (Department of Health, 1999).

1.4.2 Acute Rheumatic Fever

ARF develops between one and six weeks following an episode of GAS pharyngitis that has been left untreated in susceptible children and adolescents aged 5-15 years (Rammelkamp and Stolzer, 1961, Quinn and Federspiel, 1967). ARF is a multi-organ disease characterised by inflammation of joints, heart structures, central nervous system and skin. The revised, updated and modified Jones' criteria (Special writing group of the committee on rheumatic fever, 1992), is recommended by the American Heart Association for diagnosing ARF, although Carapetis argues for considering the 2002 – 2003 WHO criteria (World Health Organization., 2004) (Box 1.6) which enhances the diagnosis of recurrent ARF in patients with established RHD (Carapetis et al., 2005a). Symptoms do however, vary, depending on the tissues involved, although polyarthritis is the most common (Cunningham, 2000). Carditis, which is seen in 65% of patients, is the most serious complication of ARF given that ARF patients with carditis go on to develop RHD (Bland and Duckett Jones, 1951).

Box 1.6 2002–2003 WHO criteria for the diagnosis of rheumatic fever and rheumatic heart disease (World Health Organization., 2004)

Diagnostic categories	Criteria
Primary episode of RF.[a]	Two major *or one major and two minor** manifestations **plus** evidence of a preceding group A streptococcal infection***.
Recurrent attack of RF in a patient **without** established rheumatic heart disease.[b]	Two major or one major and two minor manifestations **plus** evidence of a preceding group A streptococcal infection.
Recurrent attack of RF in a patient **with** established rheumatic heart disease.	Two minor manifestations **plus** evidence of a preceding group A streptococcal infection.[c]
Rheumatic chorea. Insidious onset rheumatic carditis.[b]	Other major manifestations or evidence of group A streptococcal infection not required.
Chronic valve lesions of RHD (patients presenting for the first time with pure mitral stenosis or mixed mitral valve disease and/or aortic valve disease).[d]	Do not require any other criteria to be diagnosed as having rheumatic heart disease.
* Major manifestations	— carditis — polyarthritis — chorea — erythema marginatum — subcutaneous nodules
** Minor manifestations	— clinical: fever, polyarthralgia — laboratory: elevated acute phase reactants (erythrocyte sedimentation rate or leukocyte count) — electrocardiogram: prolonged P-R interval
*** Supporting evidence of a preceding streptococcal infection within the last 45 days	— elevated or rising antistreptolysin-O or other streptococcal antibody, or — a positive throat culture, or — rapid antigen test for group A streptococci, or — recent scarlet fever.

[a] Patients may present with polyarthritis (or with only polyarthralgia or monoarthritis) and with several (3 or more) other minor manifestations, together with evidence of recent group A streptococcal infection. Some of these cases may later turnout to be rheumatic fever. It is prudent to consider them as cases of "probable rheumatic fever" (once other diagnoses are excluded) and advise regular secondary prophylaxis. Such patients require close follow up and regular examination of the heart. This cautious approach is particularly suitable for patients in vulnerable age groups in high incidence settings.
[b] Infective endocarditis should be excluded.
[c] Some patients with recurrent attacks may not fulfil these criteria.
[d] Congenital heart disease should be excluded.

1.4.3 Rheumatic Heart Disease

RHD is characterized by permanent valve damage resulting from the cumulative effects of recurrent attacks of ARF, although initial attacks can lead directly to RHD diagnosed in patients with no recollection of ARF (Carapetis et al., 2005a, Marijon et al., 2012). More than one valve may be affected, although the most common lesion is mitral valve disease (Bland and Duckett Jones, 1951, Rashed et al., 2007). On pathological examination, the mitral valve shows a deformed appearance with thickening of the leaflets as a result of cellular infiltration during the immunological response, which is also responsible for the diminished movement of the valve. In addition, neovascularisation, the after-effect of inflammation, interstitial calcification, as well as the presence of Aschoff's nodules, which comprises degenerated collagen surrounded by activated histiocytic cells and lymphoid cells, may also be noted (Veasy and Hill, 1997, Stollerman, 1975) (Figure 1.9). The valve surface may also show ulceration and have fibrinous vegetations near the line of closure (Rashed et al., 2007). Shortening and thickening of the chordae tendineae and papillary muscles result in the so-called "funnel-shaped valve" (Stollerman, 1975). For the remaining valves, deformities rarely occur in isolation (Shrestha et al., 2012). Tricuspid valve deformities in chronic RHD are rare, although the risk of tricuspid valve regurgitation increases following successful mitral valve surgery or development of pulmonary hypertension (Katsi et al., 2012)

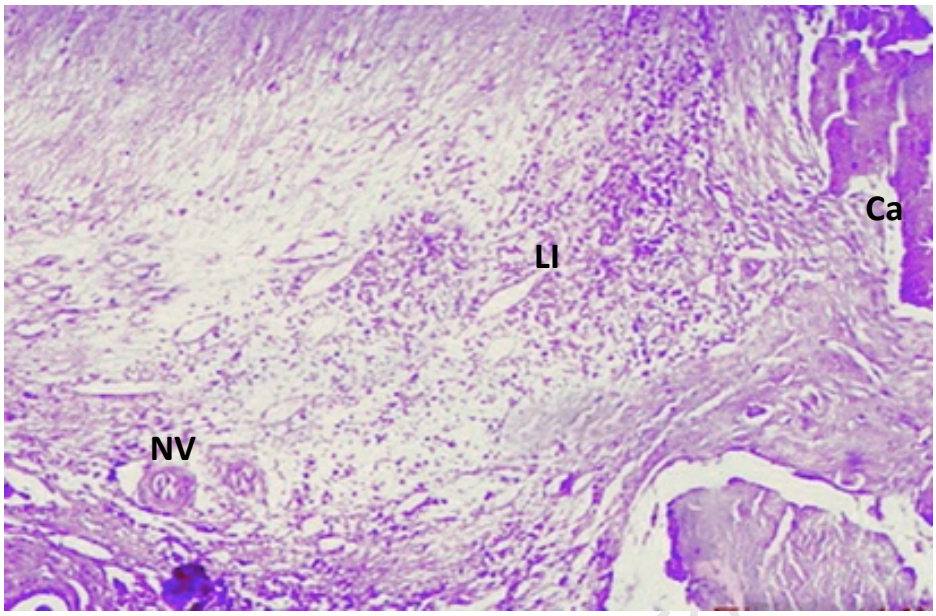


Figure 1.9. Light microscopy image of mitral valve from RHD patients undergoing surgery, showing neovascularization (NV), lymphocytes infiltration (LI) and calcification (ca) (x 90 magnification) (Rashed et al., 2007)

1.4.3.1 Echocardiographic criteria for the diagnosis of RHD

In diagnosing subclinical RHD, echocardiography has been shown to be significantly superior by as much as ten-fold as compared with traditional auscultation for a heart murmur (Figure 1.10) (Reeves et al., 2011, Marijon et al., 2012). Recently, new standardized international echocardiographic guidelines, *The World Heart Federation criteria for echocardiographic diagnosis of rheumatic heart disease,* were developed with clear definitions for the diagnosis of RHD across three categories namely, 'definite RHD', 'borderline RHD', and 'normal' using assessment by 2D, continuous-wave and color-Doppler echocardiography (Remenyi et al., 2012). Further subcategories are also defined within the 'definite RHD' diagnosis (Box 1.7).

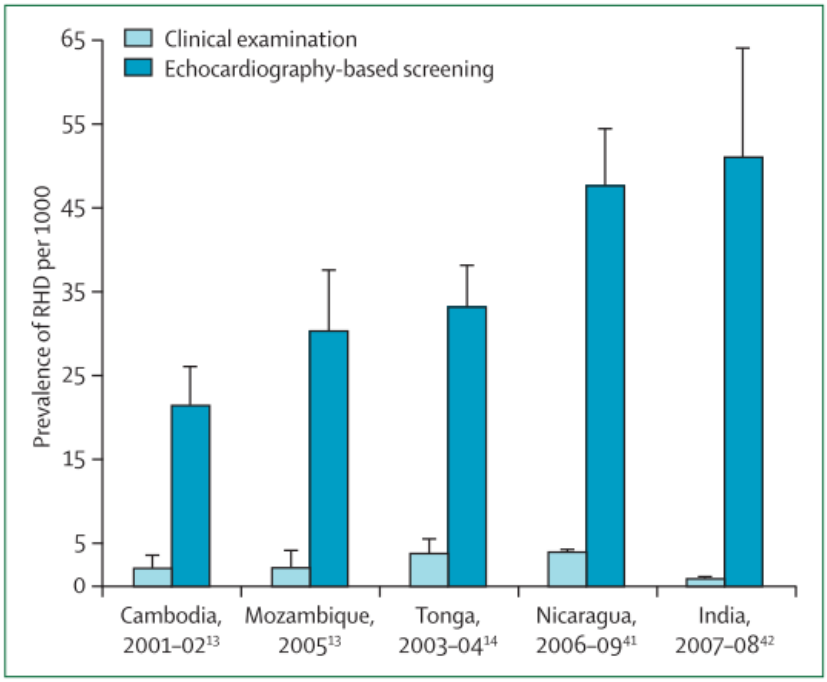


Figure 1.10. Rheumatic heart disease (RHD) prevalence rates in children: echocardiography-based screening versus clinical examination (Marijon et al., 2012).

Box 1.7 2012 WHF criteria for echocardiographic diagnosis of RHD (Remenyi et al., 2012)

Echocardiographic criteria for individuals aged ≤20 years

Definite RHD (either A, B, C, or D):

A) Pathological MR and at least two morphological features of RHD of the MV
B) MS mean gradient ≥ 4 mmHg*
C) Pathological AR and at least two morphological features of RHD of the AV‡
D) Borderline disease of both the AV and MV§

Borderline RHD (either A, B, or C):

A) At least two morphological features of RHD of the MV without pathological MR or MS
B) Pathological MR
C) Pathological AR

Normal echocardiographic findings (all of A, B, C, and D):

A) MR that does not meet all four Doppler echocardiographic criteria (physiological MR)
B) AR that does not meet all four Doppler echocardiographic criteria (physiological AR)
C) An isolated morphological feature of RHD of the MV (for example, valvular thickening) without any associated pathological stenosis or regurgitation
D) Morphological feature of RHD of the AV (for example, valvular thickening) without any associated pathological stenosis or regurgitation

Echocardiographic criteria for individuals aged >20 years

Definite RHD (either A, B, C, or D):

A) Pathological MR and at least two morphological features of RHD of the MV
B) MS mean gradient ≥ 4 mmHg*
C) Pathological AR and at least two morphological features of RHD of the AV, only in individuals aged <35 years‡
D) Pathological AR and at least two morphological features of RHD of the MV

Morphological features of RHD

Features in the MV

- AMVL thickening* ≥ 3 mm (age-specific)‡
- Chordal thickening
- Restricted leaflet motion§
- Excessive leaflet tip motion during systole

Morphological features of RHD (cont')

Features in the AV

- Irregular or focal thickening
- Coaptation defect
- Restricted leaflet motion
- Prolapse

Criteria for pathological regurgitation

Pathological mitral regurgitation
(All four Doppler echocardiographic criteria must be met)

- Seen in two views
- In at least one view, jet length ≥2 cm*
- Velocity ≥3 m/s for one complete envelope
- Pan-systolic jet in at least one envelope

Pathological aortic regurgitation
(All four Doppler echocardiographic criteria must be met)

- Seen in two views
- In at least one view, jet length ≥1 cm*
- Velocity ≥3 m/s in early diastole
- Pan-diastolic jet in at least one envelope

**A regurgitant jet length should be measured from the vena contracta to the last pixel of regurgitant color (blue or red).*

*Congenital MV anomalies must be excluded. Furthermore, inflow obstruction due to non-rheumatic mitral annular calcification must be excluded in adults. ‡Bicuspid AV, dilated aortic root, and hypertension must be excluded. §Combined AR and MR in high prevalence regions and in the absence of congenital heart disease is regarded as rheumatic. Abbreviations: AR, aortic regurgitation; AV, aortic valve; MR, mitral regurgitation; MS, mitral stenosis; MV, mitral valve; RHD, rheumatic heart disease; WHF, World Heart Federation.

1.5 EPIDEMIOLOGY OF GAS, ARF AND RHD IN SCHOOL CHILDREN

The prevalence of ARF and RHD in developed countries has shown considerable decline during the last century, largely attributed to improved living conditions and access to better healthcare (Quinn, 1989). Figure 1.11 demonstrates the declining rates of death per 100,000 due to ARF over the decades, highlighting significant milestones and achievements in medical science (Bonow and Braunwald, 2012).

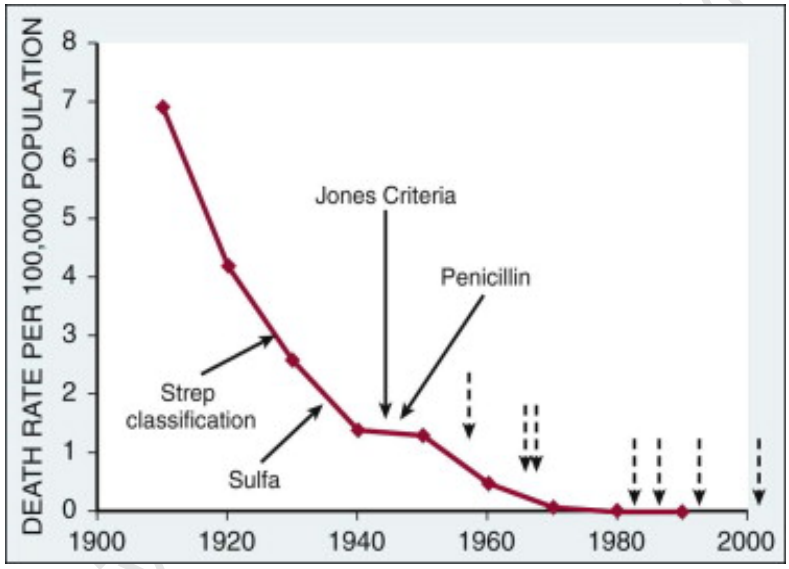


Figure 1.11. Declining rates of death due to Rheumatic Fever. Dotted arrows indicates dates of revision of Jones' Criteria or World Health Organisation reviews (Bonow and Braunwald, 2012).

In contrast, epidemiological data from developing countries, while scant, indicate the continued high prevalence of GAS-positive pharyngitis and RHD. Alarmingly, a resurgence of ARF/RHD has been observed in the former Soviet Union republics of Central Asia (Omurzakova et al., 2009). In fact, a recent review on the worldwide epidemiology of ARF and RHD identified increasing trends in the prevalence of RHD for

each WHO region in the world except for Europe (Figure 1.12) (Seckeler and Hoke, 2011); these findings are probably attributed to better ascertainment through the use of portable echocardiography, and better survival of cases of RHD.

As regards Africa though, few studies on the epidemiology of ARF/RHD are reported particularly in South Africa and efforts are underway to ascertain the burden of disease in Africa and elsewhere through initiatives such the REMEDY study (Karthikeyan et al., 2011). Recently, Sliwa et al. estimated an annual incidence of 23.5 new cases of RHD per 100,000 population aged >14 years in the areas surrounding Johannesburg. This study,

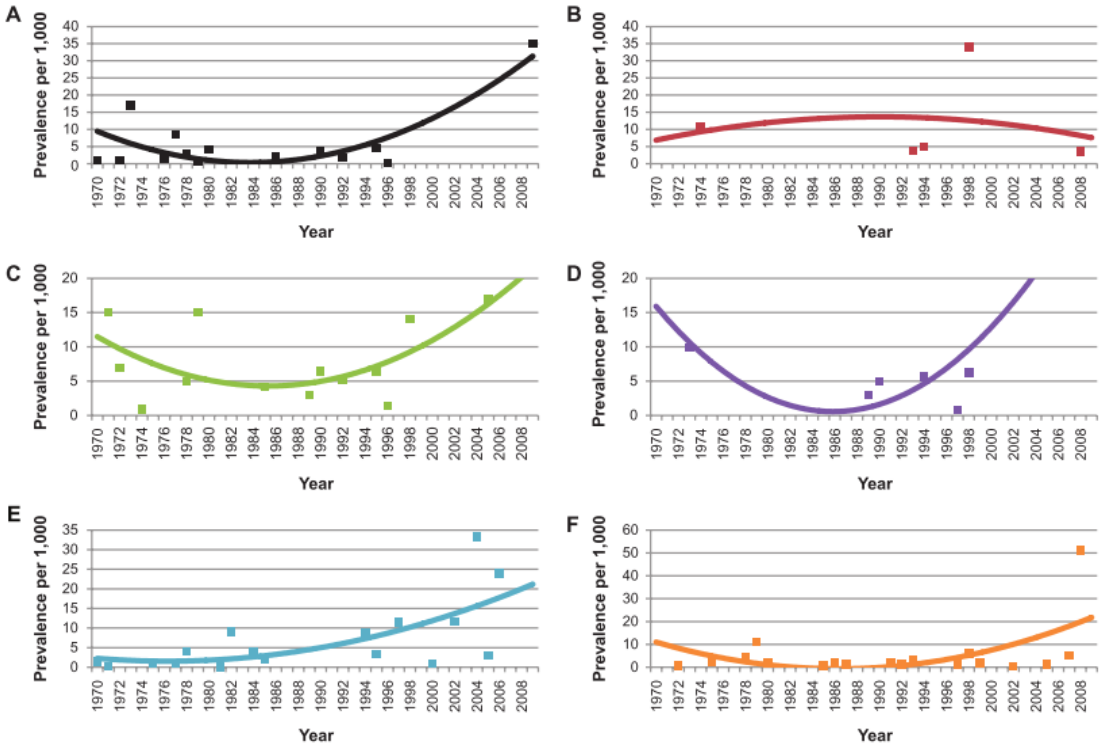


Figure 1.12. Trends of rheumatic heart disease prevalence per 1000 persons for each WHO region, A) The Americas, B) Europe, C) Africa, D) Eastern Mediterranean, E) Western Pacific, and F) Southeast Asia. Points represent reported prevalence from the literature (Seckeler and Hoke, 2011).

conducted over 24 months in a cardiac clinic of a large hospital, documented all patients with a first-time diagnosis of heart failure due to RHD; all ages of patients were represented, thus confirming that RHD is present throughout the life course (Sliwa et al., 2010).

The epidemiology of GAS pharyngitis, ARF, and RHD is considered below, with particular emphasis on school children.

1.5.1 Group A Streptococcus

1.5.1.1 Asymptomatic Pharyngeal Carriage

Asymptomatic children can be a major reservoir of pharyngeal GAS. A pooled GAS carriage prevalence of 12% (95% CI: 9% - 14%) in healthy children aged 5 – 17 years was reported in a recent review of 18 clinic- and school-based studies on streptococcal carriage in both industrialised and developing countries (Shaikh et al., 2010). Amongst the seven studies on school-aged children included in the review, the prevalence of asymptomatic carriage ranged from 10% in Sweden to 21% in Iran.

In another 16-month follow-up study conducted in community-based family medicine practices in Australia, seasonal carriage rates ranged from 8% - 16% amongst 160 randomly selected families (Danchin et al., 2007), while in a prospective surveillance study conducted over 9 months in Fiji, a GAS carriage of 6.0% was observed amongst 685 healthy children (Steer et al., 2009).

Results from India show contrasting figures; in Chennai, 8.4% of 1102 school children from overcrowded government or charity-aided schools in slum-like conditions harboured GAS (Lloyd et al., 2006) while in a rural community in Northern India, a prevalence rate of only 1.3 per cent was observed in 3385 children aged 5-15 years (Kumar et al., 2009a). Still within the region, a cross-sectional study across 4 schools in Nepal, isolated GAS from 10,9% of 350 students 5-15 years of age (Dumre et al., 2009). Elsewhere, in Grenada, a study conducted in randomly selected schools observed a GAS prevalence of 5.2% among 1388 children aged 5-15 years (Noel et al., 2005).

Data on GAS carriage from countries in Africa remain scant with only a few studies reporting on carriage. In Ethiopia, Abdissa reported a 9.7% carriage rate in pharyngeal isolates from 937 healthy participants aged 6-14 years (mean age, 11 years) (Abdissa et al., 2011). An earlier study in Tunisia documented a rate of 9.0% from throat swabs taken from 155 controls (Mzoughi et al., 2004). More recently, Sadoh reported a prevalence of almost 10% among asymptomatic school children in Nigeria (Sadoh and Omokhodion, 2007). Table 1.2 provides details of individual studies conducted outside of South Africa. Of particular interest is that a number of the studies failed to confirm the absence of recent infection by serology. Most studies though, did note the symptoms at the time of the specimen being taken.

Table 1.2. GAS carriage among asymptomatic children in countries outside of South Africa

Study	Country	Study Design	Prevalence	Serology	Symptoms
(Abdissa et al., 2011)	Ethiopia	Cross-sectional	9.7%	ND	Y
(Danchin et al., 2007)	Australia	Longitudinal	8%-16%	ND	Y
(Dumre et al., 2009)	Nepal	Cross-sectional	10.9%	ND	N
(Kumar et al., 2009b)	Northern India	Cross-sectional	1.3%	ND	Y
(Lloyd et al., 2006)	Chennai, India	Cross-sectional	8.4%	ND	Y
(Mzoughi et al., 2004)	Tunisia	Longitudinal	9.0%	UC	UC
(Noel et al., 2005)	Grenada	Cross-sectional	15.4%	Y	UC
(Sadoh and Omokhodion, 2007)	Nigeria	Cross-sectional	10%	ND	Y
(Steer et al., 2009)	Fiji	Longitudinal	6%	ND	Y

ND, not done; Y, yes; N, no; UC, unclear.

In South Africa, there is a dearth of recent studies on GAS carriage rates in school-aged children, with only four studies conducted more than 25 years ago. In a study of 12,050 school children from largely lower-socioeconomic households in Soweto, isolation rates of 5.2% were reported with a significantly higher rate of GAS isolation during the winter months and a peak incidence in fifth and sixth school grades (McLaren et al., 1975), In another study from the northern part of South Africa, contrasting carriage rates of 1.62%

and 16.8% were reported in asymptomatic Black participants from a remote traditional community and an urban setting respectively (Van Staden et al., 1982). In the same study, urban Whites had a carriage prevalence of only 3.4%. A study conducted in the late 1970s in the densely overcrowded Hout Bay community of Cape Town reported an overall prevalence of 3.6% amongst 1150 children aged 6 – 17 years (Bundred, 1986). The two schools surveyed had respective prevalence rates of 2.4% and 12%, the latter speculated to be reflective of the 10 km distance from the nearest primary health care polyclinic. Finally, a study involving mostly grade 3 school children of either mixed or Indian ancestry reported GAS carriage rates >20% in summer and <5% in spring (Ransome et al., 1983).

Few studies report on the distribution of emm types amongst asymptomatic GAS carriers; in Fiji, while not reporting specific *emm* types, Steer et al observed that 32% of GAS *emm* sequence types were shared between carriage and sore throat isolates (Steer et al., 2009). To date, only one study reports on the distribution of emm types amongst asymptomatic GAS carriers in Africa, indicating diversity in M strains (Abdissa et al., 2006).

1.5.1.2 GAS Pharyngitis

GAS-positive pharyngitis is among school-aged learners, with the peak age of incidence for GAS infections being between 5 and 15 years (WHO, 2004).

Generally, developing countries have higher prevalence rates of GAS isolated from patients with pharyngitis compared with industrialised nations, except for impoverished populations within industrialised countries (Steer et al., 2007). A recent review of 17 studies of GAS prevalence calculated a pooled prevalence estimate of 37% among children presenting with

sore throat from both industrialised and developing countries (Figure 1.13) (Shaikh et al., 2010). Of the studies included in the review, the prevalence rates ranged from 23% in the United States to 58% in a study from the Netherlands; although, on closer inspection of the Netherlands study, the isolation of GAS was actually only 32% (Dagnelie et al., 1993).

Source	Age Range, y	*N*	Setting	Country	Prevalence (%)
All ages					
Romoin et al,[15] 2005	5–12	916	Clinic	Egypt/Croatia/Brazil	33
McIsaac et al,[16] 2004	3–17	454	Clinic	Canada	34
McIsaac et al,[17] 2000	3–14	158	Clinic	Canada	35
de Silva et al,[18] 1998	3–12	137	Clinic	Sri Lanka	45
McIsaac et al,[19] 1998	3–14	94	Clinic	Canada	36
Gunnarsson et al,[11] 1997	3–15	106	Clinic	Sweden	34
Dobbs et al,[20] 1996	4–11	86	Clinic	Ireland	48
Dagnelie et al,[21] 1993	4–14	80	Clinic	Netherlands	58
Pichichero et al,[22] 1992	<18	65 463	Clinic	United States	23
Hoffman et al,[23] 1992	≤14	466	Clinic	Denmark	42
Reed et al,[24] 1990	<19	375	ED	United States	33
Reed et al,[25] 1988	2–12	136	Clinic	United States	32
Feery et al,[26] 1976	6–16	47	Clinic	Australia	45
Forsyth et al,[27] 1975	≤14	213	Clinic	United States	31
Pooled prevalence (95% CI)					37 (32–43)
Younger than 5 y					
Romoin et al,[15] 2005	2–5	894	Clinic	Egypt/Croatia/Brazil	24
Gunnarsson et al,[11] 1997	0–2	40	Clinic	Sweden	18
Feery et al,[26] 1976	0–5	30	Clinic	Australia	17
Pooled prevalence (95% CI)					24 (21–26)

ED indicates emergency department.

Figure 1.13. Prevalence of GAS Infection Among Children Presenting With Sore Throat (Shaikh et al., 2010)

In the same review, two studies from developing countries reported rates of 45% (Sri Lanka) and 33% (Egypt/Croatia/Brazil) respectively.

In a hospital-based study from Kolkata, GAS was isolated from 42 out of 100 throat swabs from patients of all ages presenting with pharyngitis, with a peak incidence observed in the 5-15 years age group (Ray et al., 2010). Elsewhere in India, a cross-sectional study

comprising 4249 children aged 5-15 years from 25 randomly selected villages in the Panchkula district of Haryana in northern India, reported respective prevalence rates for βHS and GAS of 25.7% and 2.8% from children with pharyngitis with rates of isolation being significantly higher in the winter months (Kumar et al., 2009a). In the same study, the investigators observed pharyngeal βHS and GAS carriage rates of 15.4% and 1.3% respectively.

A number of studies have documented incidence of GAS pharyngitis. Table 1.3 summarizes the respective incidence rates of acute sore throat and GAS swab-positive pharyngitis, indicating the method of ascertainment of participants.

Table 1.3. Incidence of symptomatic GAS pharyngitis among children

Study	Country	Method of Ascertainment	Incid of sore throat / 100 child-yrs	Incid of GAS pharyngitis / 100 child-yrs
(Duben et al., 1979)	(Former) Czechoslovakia	Active	8.3	3.9
(Nandi et al., 2001)	Northern India	Active	705	95
(Danchin et al., 2007)	Melbourne, Australia	Active	33	13
(Steer et al., 2009)	Melbourne, Australia	Active	162	14.7
(McDonald et al., 2006)	Darwin, Australia	Active	8	0

Incid, incidence; GAS, Group A streptococcus; yrs, yeasrs

Prevalence and incidence data on GAS pharyngitis from developing countries are largely lacking as compared with industrialised nations (Carapetis et al., 2005a), especially in

South Africa. A study conducted in Pretoria over 30 years ago on 232 unselected patients who presented with a complaint of sore throat reported an overall prevalence of 33.2% with a significant difference between rates for Blacks (45.5%) and Whites (23.2%) (van Zyl et al., 1981). No variation in rates was observed by season and the overall background carriage rate of 165 controls was 12.1% (Blacks, 16.8%; Whites, 3.4%). In another study of 112 participants aged two to nineteen years of age conducted during the summer months at a hospital serving the Black community in Bloemfontein, 42% of throat swabs cultured returned a positive GAS result (Olivier and de Graad, 1978).

1.5.2 Acute Rheumatic Fever

The ascertainment of true incidence rates of ARF is often hampered by the lack of active national control programmes to ensure adequate reporting and control through the use of registry-based systems (McDonald et al., 2005), a phenomenon also observed in South Africa (Robertson et al., 2005b).

A recent systematic review included 14 papers on the burden of ARF, observing a global distribution in the incidence rates reported (Jackson 2011); incidence and mortality rates in developed countries remain low, while for Sudan, an incidence of 826 per 10^5 amongst school children was reported, an incidence greater than 15 times the previous estimate of 50 per 100 000 children in developing countries (Carapetis et al., 2005a). Seckler and colleagues recently highlighted the global distribution of trends in the incidence of ARF, indicating increases in the Americas, Eastern Mediterranean countries and Southeast Asia (Seckeler and Hoke, 2011). However, in the same study, the apparent decrease in the

incidence of ARF reported in Africa is probably attributed to ascertainment bias in that episodes of ARF may often go unnoticed in poor countries with poor access to health care and health-seeking behaviour on the part of the patients (Karthikeyan and Mayosi, 2009).

For South Africa, anecdotal information suggests that the incidence of ARF remains quite high and in fact, South Africa may be in the midst of an ARF epidemic (Department of Health, 2002b), despite national guidelines and recommendations for antibiotic prevention of the disease (Department of Health, 1999, Department of Health, 2006). Practical experience indicates a high incidence of ARF and prevalence of RHD accounting for 2% of paediatric admissions (personal communication cited in Brink, 2004).

1.5.3 Rheumatic Heart Disease

Data on RHD show variation across different regions of the world with prevalence rates from as low as 68 per 100,000 in India to as high 3320 per 100,000 in Tonga and mortality rates from 1.2 to 2.8 per 100,000 reported in a recent review (Jackson et al., 2011). African data as regards RHD mortality remain scant, with no African studies available for inclusion into the review.

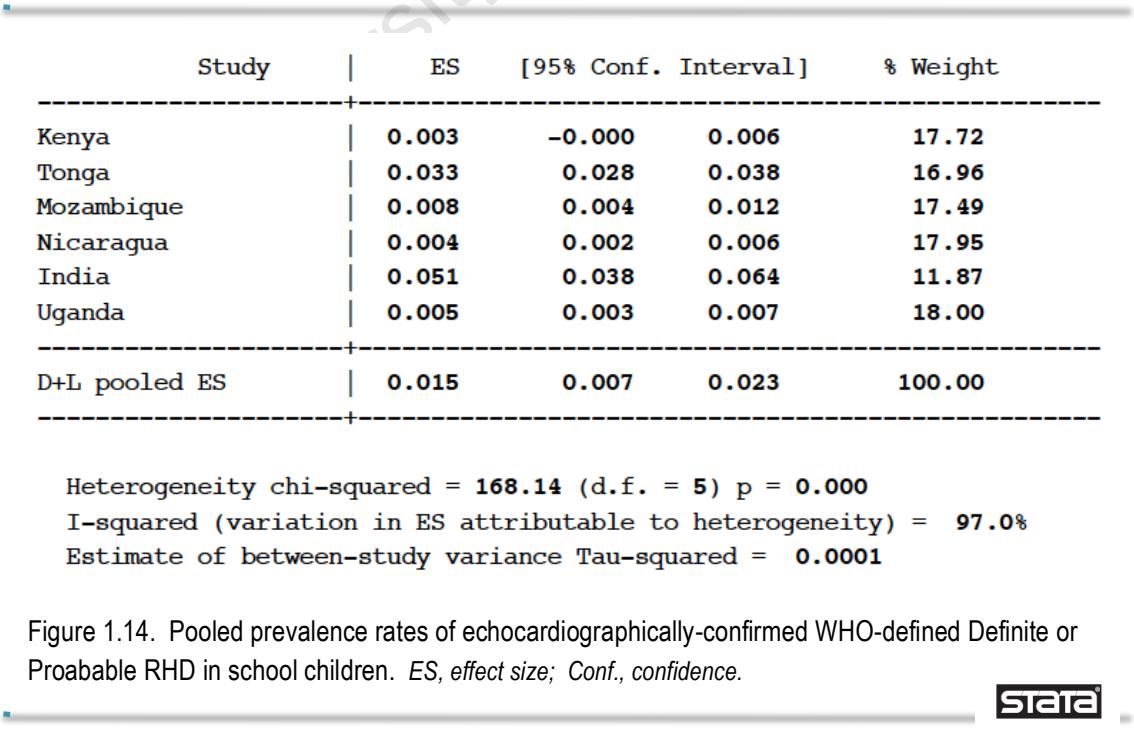
While a number of school-based studies on the prevalence of RHD have been reported, the majority of these did not employ echocardiography for screening. Instead, echocardiography was often only used to confirm clinical suspicion. Thus, current estimates may well represent an under estimation of the prevalence, given that of late, a number of reports have shown echocardiography to be a more sensitive approach for screening than auscultation, detecting up to ten times more cases than auscultation in some studies (Marijon et al., 2008).

Prevalence rates for echocardiographically-screened RHD amongst school children range from 2.7 per 1000 to 50 per 1000. Table 1.4. illustrates the prevalence rates obtained by echocardiography-based screening and the criteria used for diagnosis. When put into a meta-analysis, the pooled prevalence rate for definite or probable RHD as defined by WHO criteria (where it was possible to extract data from the original reports), is 15 per 1000, (95% CI, 7 – 23 per 1000) (Stata output, Figure 1.14).

Table 1.4. Prevalence of RHD among asymptomatic school children (as extracted from original reports) screened by echocardiography

Author	Study Date	Country	Criteria	N	pr / 1000 (95% CI)
(Anabwani et al, 1996)	1994	Kenya	"*Colour flow*"	1115	2.7 (0.6 – 7.8)
(Marijon et al., 2009)	2001 - 2	Cambodia	"*Consensus-based*"	3677	21.5 (16.8 – 26.2)
(Carapetis et al., 2008)	2003 - 4	Tonga	Modified WHO	5053	33.2 (28.7 – 38.6)
(Marijon et al., 2009)	2005	Mozambique	"*Consensus-based*"	2170	30.4 (23.3 – 37.6)
(Paar et al., 2010)	2006 - 9	Nicaragua	WHO/NIH	3151	48 (35.0 – 60.0)
(Bhaya et al., 2010)	2007 - 8	India	WHO	1059	50.0 (39.0 – 66.0)
(Beaton et al., 2012)	2010	Uganda	WHO/NIH	4,869	14.8 (7.3 – 22.3)

N, Number examined by echocardiography; pr, prevalence; CI, confidence interval



Study	ES	[95% Conf.	Interval]	% Weight
Kenya	0.003	-0.000	0.006	17.72
Tonga	0.033	0.028	0.038	16.96
Mozambique	0.008	0.004	0.012	17.49
Nicaragua	0.004	0.002	0.006	17.95
India	0.051	0.038	0.064	11.87
Uganda	0.005	0.003	0.007	18.00
D+L pooled ES	0.015	0.007	0.023	100.00

Heterogeneity chi-squared = 168.14 (d.f. = 5) p = 0.000
I-squared (variation in ES attributable to heterogeneity) = 97.0%
Estimate of between-study variance Tau-squared = 0.0001

Figure 1.14. Pooled prevalence rates of echocardiographically-confirmed WHO-defined Definite or Proabable RHD in school children. *ES, effect size; Conf., confidence.*

1.5.3.1 Auscultation-based School Screening for RHD in South Africa

There are four studies on the prevalence of RHD among school children in South Africa (Bundred, 1986, Maharaj et al., 1987, McLaren et al., 1975, Pocock et al., 1968).

Two of the studies were conducted in school children from Soweto, Johannesburg; Pocock and colleagues, who screened 428 children, reported a prevalence of between 5 and 10 per 1000 at the Southern Africa Cardiac Society Conference, (Pocock et al., 1968). The second study, conducted over 5 months in 1972, enrolled 12,000 randomly-selected children between the ages of 2 and 18 years across all grades (McLaren et al., 1975). The participants were examined by ten cardiologists, three of whom needed to have agreement on a diagnosis of RHD following an independent examination of children having an abnormal heart. An overall prevalence rate of 6.9 per 1000 was observed, although this figure went up to as high as 11 / 1000 in older children. A preponderance of females to males of 1.6:1 was observed. The third study was conducted among 1150 school children from the lower income peri-urban fishing community of Hout Bay in Cape Town (Bundred, 1986). Two schools, one being a Moravian Mission outreach project for children of the local farm labourers, participated in the survey. Using auscultation for screening, and echocardiography for confirmation of abnormal cases, the group reported a combined prevalence rate of 6.9 per 1000. The last study reported a prevalence of 1 per 1000 using the WHO-defined auscultation criteria among 4408 school children aged 4 – 18 years from the Inanda township community on the periphery of Durban (Maharaj et al., 1987).

1.6 PREVENTION OF ARF AND RHD

1.6.1 Primary Prevention of Rheumatic Fever

Primary prevention of rheumatic fever is defined as the administration of adequate antibiotic treatment in individuals diagnosed with GAS pharyngitis so as to prevent ARF (World Health Organization., 2004). A meta-analysis has provided firm evidence for a reduction in the risk of developing ARF resulting from the administration of antibiotics following a GAS pharyngitis episode, relative risk of 0.32 (95% CI = 0.21–0.48) (Robertson et al., 2005a). Subgroup analysis of penicillin alone was associated with an even more effective outcome and thus, Robertson refutes the reservations of resistance to penicillin, especially given the rarity of anaphylactic reactions.

The WHO recommended dosage for primary prevention is indicated in Box 1.8. In South Africa, the Department of Health publications, *Standard Treatment Guidelines and Essential Drugs List (EDL) for South Africa, Primary Health Care 2003 Edition (Department of Health, 2006)* and *National Guidelines on the Primary Prevention and Prophylaxis of Rheumatic Fever and Rheumatic Heart Disease for Health Professionals at Primary Level (Department of Health, 1999)* provide the recommended management strategies. Upon diagnosis, a single dose of benzathine benzylpenicillin should be administered intramuscularly according to the patient's weight as follows: less than 15 kg, 300 000 IU; 15– 30 kg, 600 000 IU and over 30 kg and adults 1.2 MU.

Box 1.8 Primary prevention of rheumatic fever: recommended treatment for streptococcal pharyngitis (World Health Organization., 2004) .

Antibiotic	Administration	Dose	Comments
Benzathine benzylpenicillin	Single intramuscular injection	1 200 000 units intramuscularly; 600 000 units for children weighing <27 kg.	Preferable to oral penicillin because of patient adherence problems.
Phenoxymethyl penicillin (Penicillin V)	Orally 2–4 times/day for 10 full days	Children: 250 mg bid or tid. Adolescents or adults: 250 mg tid or qid, or 500 mg bid.	Penicillin resistance by group A streptococci has never been reported.
Amoxicillin	Orally 2–3 times/day for 10 full days	25–50 mg/kg/day in three doses. Total adult dose is 750–1500 mg/day.	Acceptable alternative to oral penicillin because of the taste.
First-generation cephalosporins[c]	Orally 2–3 times/day for 10 full days	Varies with agent.	Acceptable alternative for oral penicillin.[d]
Erythromycin ethylsuccinate	Orally 4 times/day for 10 full days	Varies with formulation. Available as the stearate, ethylsuccinate, estolate or base.	Alternative drug for patients allergic to penicillin. Should not be used in areas where group A streptococci have high rates of macrolide resistance.

[a] Modified in part from (*24*).
[b] In some countries, macrolides have been approved for an abbreviated course of therapy (shorter than 10 days), but the efficacy of this treatment is controversial and it cannot be recommended at present. Also, trimethoprim, sulfonamides and tetracyclines are not effective antibiotics for eradicating Group A streptococci and are not indicated for the primary prevention of RF.
[c] These agents should not be used in patients who have had immediate-type hypersensitivity to beta-lactam antibiotics.
[d] This has been used in some patients who have either a poorly documented history of penicillin allergy, but should not be used for patients with immediate hypersensitivity reactions to penicillin (e.g. anaphylaxis or hives). About 5% of those who have even a mild allergic reaction to penicillin may also have a reaction to cephalosporins.

1.6.2 Secondary Prevention of Rheumatic Heart Disease

Secondary prevention refers to the uninterrupted administration of antibiotic therapy for an extended period of time following the diagnosis of ARF so as to prevent recurrences of ARF and progression to RHD. Box 1.9 details the WHO regime for secondary prevention of ARF (World Health Organization., 2004).

Box 1.9 Antibiotics and dosage used in the secondary prophylaxis of RF **(World Health Organization., 2004)**

Antibiotic	Mode of administration	Dose
Benzathine benzylpenicillin	Single intramuscular injection every 3–4 weeks.	For adults and children ≥30 kg in weight: 1 200 000 units. For children <30 kg in weight: 600 000 units.
Penicillin V.	Oral.	250 mg twice daily.
Sulfonamide (e.g. sulfadiazine, sulfadoxine, sulfisoxazole).	Oral.	For adults and children ≥30 kg in weight: 1 gram daily. For children <30 kg in weight: 500 mg daily.
Erythromycin.	Oral.	250 mg twice daily.

Category of patient	Duration of prophylaxis
Patient without proven carditis.	For 5 years after the last attack, or until 18 years of age (whichever is longer).
Patient with carditis (mild mitral regurgitation or healed carditis).	For 10 years after the last attack, or at least until 25 years of age (whichever is longer).
More severe valvular disease.	Lifelong.
After valve surgery.	Lifelong.

1.6.3 Current Standard of Care in South Africa

National guidelines exist for the management of upper respiratory tract infections and the prevention of rheumatic fever (Department of Health, 1999). In South Africa, ARF was listed as a notifiable condition in 1989, with notification commencing in 1990. Although previously included, the initial diagnosis of RHD was removed and only recently, moves are afoot to have it reinstated on the list. A recent evaluation of rheumatic fever notification showed a fall in the number of RF cases and inconsistencies in the reporting mechanisms at various levels (Nkgudi et al., 2006). Furthermore, a study by Robertson et al showed a lack of awareness of the guidelines amongst health practitioners (Robertson et al., 2005b).

The current standard of care targets two populations: children aged 3-15 years for primary prevention and children up to 21 years/adults up to 35 years for prophylaxis of ARF/RHD. Patients aged 3-15 presenting with a sore throat are treated with penicillin if there are no signs of viral infection. In children with a previous history of ARF, penicillin treatment is continued until she/he attains 21 years of age. Those patients with established RHD remain on penicillin treatment until 35 years old. Oral erythromycin is administered to patients allergic to penicillin. All antibiotics are provided by the state health department free of charge.

1.7 SUMMARY AND CONCLUSIONS OF THE LITERATURE REVIEW

There has been a considerable amount of research on the pathogenesis of RHD as regards the host, agent and disease with elucidation of the aetiology and mechanisms involved in the disease process; however, a few questions remain unanswered.

1. Much is known about the social factors and the microbial agent that predispose to ARF. However, while it is widely accepted that a genetic effect may play a role in susceptibility to contracting ARF, the size of the genetic effect is unknown. Thus, there is a need to quantify the heritability of ARF in order to determine whether additional studies, such as whole genome scans, are justifiable.

2. Current data on GAS carriage rates among asymptomatic school children in South Africa remain scant, with no school-based studies undertaken across the complete spectrum of age groups. Recently, advancement in molecular methods has enabled the characterisation of GAS strains through M-typing of the *emm* gene. There is a need to document GAS carriage in school children of all ages, which, together with molecular characterisation of strains harboured in the pharynx of carriers, will help to ascertain the extent to which disease strains are prevalent amongst carriers. This information could potentially contribute to the development of the putative vaccine, and the monitoring of its efficacy within the South African population.

3. There is considerable heterogeneity amongst epidemiological studies on GAS pharyngitis in terms of participant selection, study setting and duration of enrolment. Few studies employed a passive surveillance approach where

participants are enrolled only at the time of presenting to the clinic or health facility, thereby reducing the risk of selection bias. Furthermore, few studies extend much beyond a year in duration, making it difficult to make conclusive judgements on seasonality.

An understanding of the incidence of GAS pharyngitis among children within a local context is crucial to inform appropriate primary prevention measures. Given that there exists no data on the incidence of GAS pharyngitis among children with pharyngitis attending primary health care clinics in South Africa, a prospective surveillance study of sufficient duration (> 3years) is required.

4. Most of existing clinical prediction rules (CPRs) were developed in industrialised countries with none having been evaluated in sub-Saharan Africa. It is preferable that a CPR is validated within a local context, especially given the poor performance of CPRs in populations different from those in which they were developed. Thus, there is a need to test the generalizability of currently recommended CPRs within the South African context and, in the event of poor performance thereof, to develop an appropriate CPR from local data. A robust CPR will facilitate the primary prevention of ARF.

5. There is no contemporary studies of the prevalence of RHD among asymptomatic school children in South Africa. The use of echocardiography in screening may reveal the true burden of asymptomatic disease, and assist in the development of preventative programmes in the country.

1.8 SCOPE AND OBJECTIVES OF THE THESIS

1. To identify and summarize all studies reporting on the concordance of ARF and RHD in monozygotic compared to dizygotic twins in order to derive quantitative estimates of the size of the genetic contribution to the risk of the condition.

2. To determine the prevalence of group A streptococcal (GAS) isolates among asymptomatic children in primary and secondary school-aged children (asymptomatic carriage).

3. To describe the prevalence and incidence of GAS among 3- to 15- year old children with pharyngitis living in the Vanguard community (Bonteheuwel/ Langa), Cape Town, South Africa.

4. To evaluate the sensitivity and specificity of existing clinical prediction rules when applied to patients with GAS pharyngitis in our setting.

5. To develop a clinical prediction rule for diagnosis of GrAS throat infection that is valid for children aged 5-15 years in the primary care setting within the South African context.

6. To determine the prevalence of echocardiographic RHD in school children.

2 RATIONALÉ AND DESIGN OF THE STUDIES

2.1 THE STOP RHD A.S.A.P. PROGRAMME IN SOUTH AFRICA

This section have been published in part in the following peer-reviewed article: ME Engel, L Zühlke, K Robertson. A.S.A.P. Programme in Rheumatic Fever and Rheumatic Heart Disease: Where are we now in South Africa? *SA Heart* 2009; 6:270-3

In October 2005, the Pan African Society of Cardiology (PASCAR) convened the 1st All Africa Workshop on Rheumatic Fever (RF) and Rheumatic Heart Disease (RHD) in Drakensberg, South Africa. Attended by delegates from various parts of Africa and the rest of the world, the event concluded with the adoption of the Drakensberg Declaration, a statement proposing an action plan targeting RF and RHD in Africa (Mayosi et al., 2006).

Four key areas had been identified as needing attention: awareness, surveillance, advocacy, and prevention and the programme was thus aptly named the A.S.A.P. Programme (Box 2.1). Essentially, the A.S.A.P. Programme aims to effect simple strategies within a comprehensive approach for RF/RHD control, beginning with the establishment of sentinel or demonstration sites across the continent. Awareness-raising is concerned with maximising case detection in communities through educating health professionals and members of the public about the signs and symptoms of RF and RHD as well as about the preceding streptococcal pharyngeal and skin infections. Success of this aspect of the programme requires an evaluation of different strategies of delivering key audience-appropriate health messages through various media. The surveillance component serves to

Box 2.1 Strategy of the A.S.A.P Programme in RF and RHD

- raise public and health professional awareness
- establish surveillance systems
- advocacy for increased resources for treatment
- promoting the prevention of RF/RHD in African nations

address the lack of reliable prevalence data and the absence of reports on RF outbreaks from developing countries (Robertson et al., 2006). The approach requires demonstration sites to create and maintain RF/RHD registers, conduct prospective RF incidence and cross-sectional RHD prevalence surveys, and establish the epidemiology of streptococcal throat and skin infections. Advocacy at the level of government is essential to reverse the trend of rising numbers of cases of RF/RHD in developing countries (Carapetis et al., 2005b). Admittedly, the global burden of other diseases tends to overshadow RF/RHD, but it is hoped that together with the surveillance component, increasing attention will be placed on the devastating effects of RF/RHD on the health of children worldwide (Robertson et al., 2006). Finally, the A.S.A.P. Programme proposes a focus on primary and secondary prevention, both for which there is sufficient evidence for their efficacy and cost-effectiveness (Robertson et al., 2005a, Manyemba and Mayosi, 2003, Karthikeyan and Mayosi, 2009).

The A.S.A.P programme collaborates with other similar programmes of the World Heart Federation (WHF) globally, and has also been adopted by the Inter Academy Medical

Panel as one of six global programmes. Following this meeting, there has been renewed interest among cardiologists, other medical and public health professionals, the community and most importantly, government to be proactive in combating RF and RHD.

2.1.1 The Establishment of the Vanguard Community Demonstration Site

Brief overview

In South Africa, the A.S.A.P. Programme has designated the Vanguard Community in Cape Town as its demonstration site. The area comprises two peri-urban suburbs of Cape Town, namely Bonteheuwel and Langa, which lie on either side of the Vanguard Road (thus 'Vanguard Community) approximately 17 kilometres from Cape Town city centre, Figure 2.1. Langa is the oldest township, having been officially opened in 1927 to accommodate black people forcibly removed from other areas of the Cape Peninsula. It is characterized by a mixture of household units, ranging from brick structures to informal

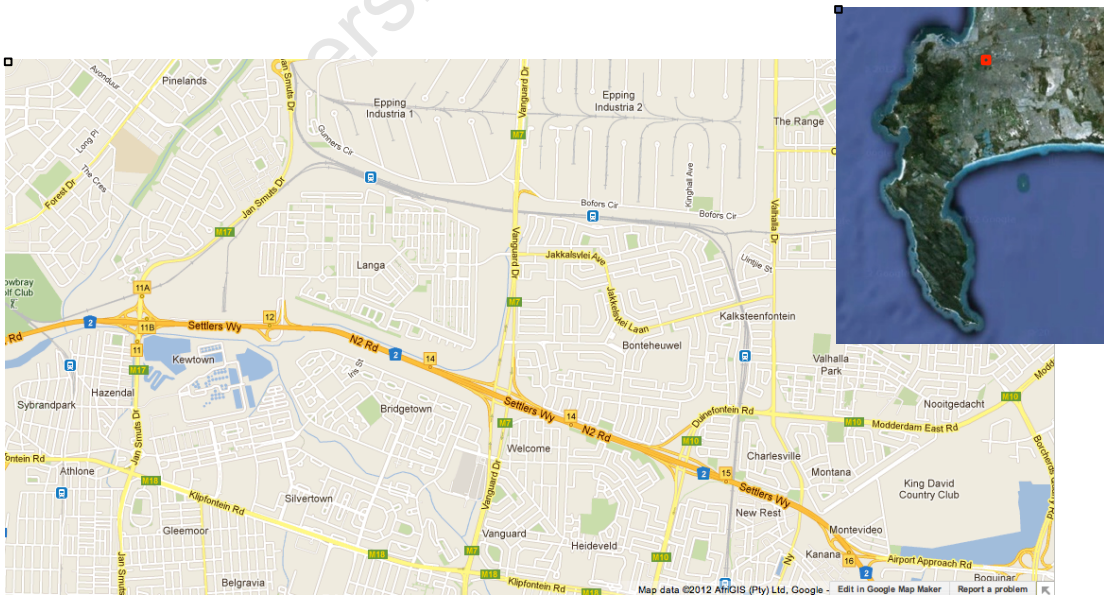


Figure 2.1. The Vanguard Community Demonstration Site

dwellings located on vacant municipality property. Bonteheuwel is a coloured township created in the 1960s as a repository for coloured people subjected to forced removals under the Group Areas Act. Both Bonteheuwel and Langa are faced with major challenges of poverty such as crime, unemployment and overcrowding. To date, the A.S.A.P. team has been conducting awareness-raising presentations amongst parents, teachers and community members. In addition, we have held seminars for health professionals to enable them to recognize and diagnose streptococcal sore throat, and make valid management decisions.

Customisation of the mobile vehicle

The recent acquisition of a customized mobile echo-surveillance unit has enabled the launch of the surveillance component of the programme, where school-aged participants can be screened for the prevalence of RHD (Figure 2.2).

Figure 2.2. The customised clinic used for screening school children

2.1.2 The Study Population

At last estimate in 2001, the South African population was 40,8 million of whom 4.5 million reside in the Western Cape (Statistics South Africa, 2003); population estimates for the Bonteheuwel and Langa are around 55,707 and 49,667 respectively living in 26,913 households. (Statistics South Africa, 2003). The communities are considered to be of lower socioeconomic status and comprise mainly black African (Langa) and people of mixed ancestry (Bonteheuwel). They are plagued with social problems such as poverty, unemployment, crime, drug and alcohol abuse, poor housing and overcrowding. Informal dwellings with limited, or no access to ablution facilities and running water, comprise 11,5% and 49,7% of the housing units in Bonteheuwel and Langa, respectively. Table 2.1 summarises characteristics of the two communities based on the 2001 census data (Statistics South Africa, 2003).

Table 2.1 Vanguard Demonstration Site: Demographic characteristics with respect to suburb.

Parameter	Bonteheuwel			Langa			*Chi*^{2}
	Count	Total	%	Count	Total	%	
Male school learners	7012	13869	*50,6%*	4501	9439	47,7%	<0.05
High School completed	951	33357	*2,9%*	701	32522	2,2%	<0.05
Non-brick dwelling	1303	11368	*11,5%*	7729	15545	49,7%	<0.05
Water in dwelling	9561	11373	*84,1%*	5498	15548	35,4%	<0.05
Electricity	11322	11373	*99,6%*	10301	15548	66,3%	<0.05
Employment	14911	22549	*66,1%*	13118	25862	50,7%	<0.05
Household income <US$ 200 /month	4107	11368	*36,1%*	11119	15545	71,5%	<0.05

The two areas contrast considerably as regards some of the indicators of socio-economic status; employment is around 58% of the adult population with more than half of the households having no income or an income of less than US$200 per month.

Approximately 20% of the population census is 5 to 15 years of age (Table 2.2); school attendance is compulsory and reported to be relatively high (Mr Paulsen Personal Communication).

Table 2.2: Age distribution amongst Vanguard children

Age in years	Frequency	Percentage
5	1827	*9.1*
6	1863	*9.3*
7	1893	*9.5*
8	1697	*8.5*
9	1860	*9.3*
10	1888	*9.4*
11	1890	*9.5*
12	1879	*9.4*
13	1764	8.8
14	1646	8.2
15	1787	8.9
Total	**19994**	**100**

Mortality data for 2001 for the whole of Cape Town and for the Central sub-districts (including Langa) and Tygerberg West (including Bonteheuwel) reveal that the leading causes of mortality in the sub-districts are HIV/AIDS, homicides, tuberculosis, ischaemic heart disease, road traffic accidents, and diabetes. Among infants low birth weight and lung disease, HIV/AIDS, diarrhoea, and lower respiratory infections are leading causes of death. The infant mortality rate per 1000 live births in 2002 was 31 and 20 respectively for the Vanguard Community Health Centre and Langa Clinic.

2.2 GENETICS STUDY: SYSTEMATIC REVIEW OF TWIN STUDIES ON ARF AND RHD

2.2.1 General Description of the Study

Systematic reviews use a rigorous method to summarise data from a collection of studies, specifically giving increased power to detect an association between risk factors and the outcome of interest. Using the systematic review method, we investigated population-based twin studies on ARF and RHD for evidence for a genetic effect in disease susceptibility and/or progression. The systematic review process was based on the guidelines set out in the Cochrane Handbook for Systematic Reviews (Higgins et al., 2009), the methods of the Human Genome Epidemiology Network (Little and Higgins, 2006). and of those of the PRISMA Statement (Moher et al., 2009) (See Appendix 10.1).

2.2.2 Specific Objective of the Genetics Study

Following the identification of all primary studies reporting on the incidence of ARF and the prevalence of RHD in monozygotic and dizygotic twins, we determined the extent to which the variation of ARF and RHD between monozygotic and dizygotic twin pairs is due to genetic effects.

2.2.3 Methods of the Systematic Review

The methods for the *systematic* review are described in detail in Chapter 3: Study One.

2.3 CLINIC-BASED STUDY: VANGUARD, NETREG AND LANGA HEALTH CENTRES

2.3.1 Study Design and Setting

We conducted this prospective observational study in the paediatric outpatient departments of the three primary health care clinics serving the Vanguard community. The provincial authority, called the Metro District Health Service, delivers all primary health care services to the people of Cape Town, of which the Metropole is divided into 8 sub-districts. The Vanguard Community Health Centre, which is situated on the border of Bonteheuwel and Langa, opened in June 2000 and has 24-hour access with comprehensive medical services including emergency facilities, obstetrics and gynaecology and dentistry.

The Netreg and Langa community clinics are based within the Bonteheuwel and Langa communities respectively, providing vaccination, TB and HIV treatment amongst other services. There is a dedicated child health service that is integrated into the rest of the health service. Clinical nurse practitioners run the well baby clinic daily and a paediatrician from Red Cross War Memorial Children's Hospital visits once a week to deal with major problems. Family planning services are provided at flexible hours to accommodate working mothers. In addition, there are ten private general practitioner clinics in this area, who did not participate in the study. To our knowledge, there were no concurrent community- or media-based interventions regarding sore throat health-seeking behaviour being administered.

2.3.2 Specific Objectives of the Clinic-based Study

- To describe the prevalence and incidence of throat isolates of GAS among 3- to 15-year-old children with pharyngitis living in the Vanguard community of Cape Town.
- To develop a clinical prediction rule for diagnosing GAS pharyngitis that is valid for the South African population.

2.3.3 Sampling Procedures

Study participation was offered to all unscheduled, walk-in paediatric patients aged 3 – 15 years who presented with symptomatic pharyngitis seeking medical care at one of the three community clinics serving the Vanguard area (Section 2.1). Patients were first triaged by clinic staff and then seen by our study nurse. We excluded patients who had received a recent prescription of antibiotics, the last dose of which was taken within 30 days prior to enrolment, or were unable or unwilling to participate. The clinic-based studies presented in this thesis used the same participants (Study 3, chapter 5; Study 4, chapter 6).

2.3.4 Ethics Matters

The study was performed with the approval of the University of Cape Town's institutional review board and the relevant department of health, and informed consent was obtained in writing from a parent or legal guardian of each participant. The consent forms were provided in the local languages of Afrikaans, English and isiXhosa. In addition, children aged 8 years and older were required to provide assent (See Appendix 10.2).

Potential Risks

According to the guidance provided by the Office of Human Research Protections, participation in this study involves a research activity that presents no more than minimal risk for the volunteers (US Department of HHS.) Taking a throat swab is a routine clinic procedure; throat swabbing may lead to brief gagging and could potentially induce vomiting. The risks of a breach of confidentiality of data are minimal given our safeguards and the nature of the study.

Known Potential Benefits

Participants in this study were examined by the study research nurse; all management decisions, however, were made by respective clinic staff based on clinical assessment. While patient management decisions were not dependent on microbiological information, the results of the throat swab were, nevertheless, made available by the study coordinator to the clinic staff as soon as possible. Participants were not paid for participating in the study.

2.3.5 Summary of Study Evaluations

The research nurse attended a training session in clinical assessment and procedures under the guidance of the study clinician. At each clinic, the nurse prospectively collected basic demographic information including recent antibiotic prescription history onto the study case record form (See Appendix 10.3), conducted a physical examination and, obtained a throat swab specimen for microbiological processing in the microbiology laboratory within 24 hours as per a standard protocol. The following signs and symptoms were assessed

(based on on the best-performing clinical prediction rules in an Egyptian prospective study (Steinhoff et al., 2005), with the addition of "absence of candidiasis".

- Cough (a negative point against the diagnosis of GAS)
- Rhinorrhoea (a negative point against the diagnosis of GAS)
- Hoarseness (a negative point against the diagnosis of GAS)
- Observed fever (T> 38ºC)
- Tonsillar erythema
- Tonsillar swelling
- The presence of exudates on the pharynx
- The presence of exudates on the tonsils
- The presence of oro-pharyngeal candidiasis (a negative point against the diagnosis of GAS)
- Tenderness of an anterior cervical node on palpation
- The presence of an anterior cervical lymph node greater than 1.5cm in diameter
- Rash (a negative point against the diagnosis of GAS)

These data informed the results of Study 3 (*Longitudinal Study of GAS pharyngitis in the Vanguard Demonstration Site*) and,

Study 4 (*Streptococcal pharyngitis in children presenting with sore throat: evaluation of existing clinical prediction rules and development of a new diagnostic prediction rule*) as reported in this thesis.

2.3.6 Specimen Collection, Transport and Handling

Collection of specimens

All throat swab specimens were collected by the study nurse according to a standard procedure. Sterile individual cotton swabs were used for all cultures. For each throat specimen, a single swab was slowly swiped across one tonsil or tonsillar fossa, then across the posterior pharynx, and finally across the opposite tonsil or tonsillar fossa with care taken not to touch the tongue or the mouth with the swab. Swabs with liquid media tips were used (Culturette or equivalent). The standard operating procedure for collection of throat specimens is found in Appendix 10.4.

Transport of specimens

Swabs collected at clinical sites were transported to the National Health Laboratory Service (NHLS) microbiology laboratory at Groote Schuur Hospital for processing on the same day, with specimens kept in an insulated bag for transport.

Handling of specimens

Swabs were received by the microbiology laboratory and logged according to routine procedures. Briefly, specimens were plated onto 4% sheep blood agar plates in a standard fashion. Effort was made to place specimens in an incubator no later than 4 hours from the time of collection. Time of collection and time of plating were recorded. Plates were inverted and incubated anaerobically at 35°C. After 18 to 24 hours, the plates were read by the microbiology technician, under the supervision of the study microbiologist. All cultures of beta-haemolytic colonies were further identified by Gram stain, catalase, and sero-grouping, as appropriate. A single colony was picked off with a sterile wire loop, and

sub-cultured for purity. Pure colonies of beta-haemolytic streptococci identified as group A, C or G were removed from the plates in a sterile fashion and placed in trypticase soy broth with glycerol for storage.

Laboratory results were downloaded from the database of the NHLS laboratory. Reports containing the microbiologic data indicating the presence or absence of beta haemolytic streptococci (group A, C or G in particular) and the storage identification number were affixed to the case report form (CRF) for simultaneous entry into the database. Specially designed batch-flow sheets were used to track the specimens and forms from the time of screening to filing after data entry was completed.

2.3.7 Data Handling And Record Keeping

Source documentation comprised information initially captured on a paper CRF at the clinic and laboratory reports extracted from the NHLS laboratory's website. Forms were filed in individual patient folders in the Mayosi Research Group's data entry centre in a fire-proof lockable cabinet.

Data-entry was performed by research assistants based in the Department of Medicine at the University of Cape Town. A purpose-designed database (Epi Info™ software package) was used for data management and analysis. All data were double-entered (i.e., entry performed independently on two separate occasions) into the database once all the source documents had been obtained. Copies of the source documents were maintained in the participant's medical chart or study file at the site, and were readily available for review. Data integrity checks were carried out on a regular basis including folder audits at both the

centre and the clinics as well as database comparisons of the double-data entry. In addition, the project was subjected to external monitoring as mandated by the sponsor.

2.3.8 Statistical Considerations

2.3.8.1 Outcome Measures

- The proportion of children with pharyngitis from whom GAS was isolated.
- The minimal incidence of GAS pharyngitis in children based on annual cases per 100 children in the catchment area.
- The age-, gender- and suburb-specific minimal incidence rates of GAS pharyngitis in children living in the catchment area
- Identification of the clinical signs and symptoms of pharyngitis which are most strongly associated with GAS infection.

2.3.8.2 Sample Size Considerations

Epidemiology of Streptococcus pyogenes pharyngitis

The number of cultures positive for GAS was expected to be directly proportional to the number of participants enrolled in the study. Given the surveillance system that we were to put in place to identify as many of the pharyngitis cases as possible, the incidence of GAS pharyngitis was based on our estimate of the number of children that would seek care compared to the most recent census data for the relevant sector(s) of Cape Town.

According to most recent census data, the population of the target area was approximately 100,000, of which we estimate that approximately 20,000 were children aged 3-15 (Statistics South Africa, 2003). Based on a 30% recovery rate, 420 cultures positive for GAS were expected each year. The study included only those who were symptomatic and presented to the community health centre with sore throat.

Clinical Prediction Rule development

Given the expected prevalence of GAS in patients with pharyngitis of 30%, and to demonstrate a 95% sensitivity of our prediction rule with a 95% confidence interval of 91% to 99%, we needed 115 GAS cases for which at least 383 patients needed to be recruited. Thus, our chosen sample size was set at 400 participants to create the CPR. Information on a further 400 participants was desirable to validate the proposed CPR. The sample size required for the derivation and validation of the CPR was therefore 800 participants.

2.3.8.3 Analysis Plan

Demographic data and a summary of all clinical signs and symptoms recorded at enrolment are presented descriptively for all children enrolled as well as for those with positive cultures for GAS. Based on data from a South African study conducted in a different province which found an overall GAS positivity of 33.2% in patients presenting with sore throat (van Zyl et al., 1981), we estimated that 420 positive cultures will be

obtained per annum. In addition, the overall annual rate of incident cases of GAS pharyngitis is presented according to gender, age and ethnicity using an estimate of the number of children three to fifteen years old in the catchment areas based on the most recent census. Significance was accepted at the two-sided level of 0.05.

Statistical Analysis used in the creation of the Clinical Prediction Rule

The reference standard in this study for diagnosis of GAS throat infection is a throat swab culture. A chi-squared test for association between each clinical symptom and sign and the result of throat swab culture were calculated together with the odds ratios and accompanying 95% confidence intervals for each sign and symptom. A multivariate logistic regression model was constructed incorporating variables with evidence of association with throat swab culture result on univariate analysis. The weighting of each variable in the final model was used to determine the weighting of each criterion in the CPR. We had expected the CPR to be derived based on the first 400 cases with a further 400 participants to be enrolled to validate the rule with no interruption between recruitment and validation, given there being no need to make any changes in methodology.

2.4 SCHOOL-BASED STUDY: SCREENING FOR GAS AND RHD

2.4.1 Study Design and Setting

We conducted this cross-sectional school-based study amongst healthy learners attending primary and secondary schools within the Vanguard Demonstration site of the A.S.A.P. Programme (Section 2.1.) from January 2008 until March 2012 following an initial pilot study conducted at a primary school within the research area, to establish the suitability of the protocols in May 2007. Schooling is compulsory and free, and uptake is estimated to be around 90% (Mr Paulsen Personal Communication). A school health service operates within the community, having one of its focuses as "*an assessing the health needs of the school community*" (Department of Health, 2002a). These are mainly directed at learners entering the school system at grades 0 and 1 to identify barriers to learning. Assessments thus include hearing assessment, vision screening, speech impairment and physical examination for gross locomotor dysfunction, oral health checks, anthropometric assessment, and on occasion, identifying and responding to intentional injuries and child abuse, and mental health assessments. No screening is done for RHD as part of the school health system.

2.4.2 Specific Objectives of the Population-based Study

- To determine the prevalence of echocardiographic RHD in school-aged children living in the "Vanguard" Area (Langa and Bonteheuwel) of Cape Town.
- To describe the prevalence of pharyngeal group A streptococcal carriage amongst asymptomatic children in primary and secondary school-aged school children in Cape Town

2.4.3 Sampling Procedures

Study participants were selected from all primary and secondary schools within the Vanguard Demonstration site. We employed an adaptation of Probability Proportional to Size (PPS) Cluster Sampling (European Social Survey Education Net.) using classrooms as the sampling frame. PPS has various advantages over simple random sampling including an assurance of spread of participants across all strata (in this case, grades and area) and eliminates the need for finding replacements in the event of learners not turning up for the examination. The random selection of the classrooms ensures control for selection bias.

Using the Department of Education's database, we compiled a list of all the schools and their respective enrolment figures in the area. Figure 2.3 shows the distribution of schools in Langa and Bonteheuwel.

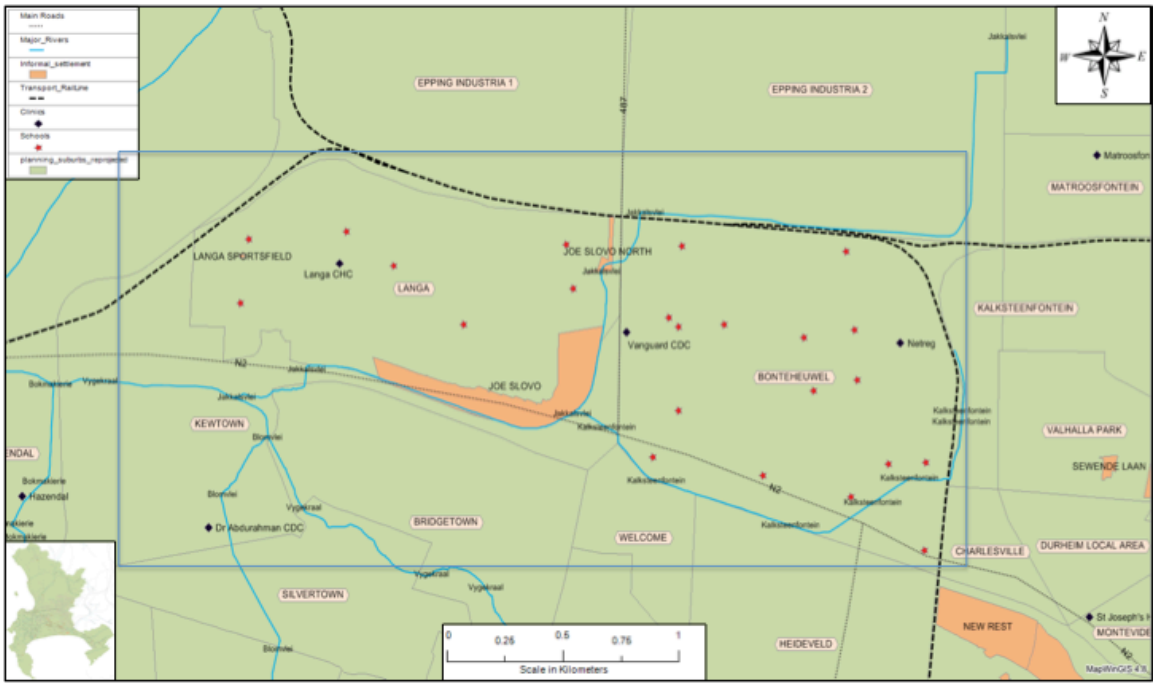


Figure 2.3. Primary and high schools within Langa and Bonteheuwel

Schools were approached individually and through principals' fora to glean interest in participating in the study, after which a final list was compiled. Class lists were used to determine the number of learners enrolled in each school.

The sampling method consisted of three stages:

1. Stratification by municipality
2. Stratification by grade
3. Simple random selection

Stage 1: Stratification by municipality

The sample size within each municipality of Langa and Bonteheuwel was calculated in proportion to the number of learners in each stratum

Stage 2: Stratification by grade

Within each stratum (municipality), the sample size within each grade was in proportion to the average number of children in the grade (sub-stratum)

Stage 3: Simple random selection

Within each municipality and grade, according to the average class size in the grade, we calculated the number of classes needed (rounding up where necessary). Classrooms were then randomly selected within a grade, regardless of school. All eligible learners within a selected classroom were invited to participate, and the parents/caregivers were asked to sign a consent form (See Appendix 10.5).

Participants enrolled in Study Two: *Carriage Rates And Genotypic Characteristics Of Group A Streptococci In Asymptomatic School children Of The Vanguard Communities Of Cape Town, South Africa* as reported in this thesis was enrolled from within the larger surveillance screening project by convenience sampling over the three-year period from February 2009 – November 2011.

2.4.4 Ethics Matters

The study was performed with the approval of the University of Cape Town's institutional review board and the relevant departments of health and education. Informed consent was obtained in writing from a parent or legal guardian of each participant following an in-class demonstration of the examination procedures in a relaxed atmosphere. Information brochures and consent forms were provided in the local languages of Afrikaans, English and isiXhosa. In addition, children aged 8 years and older were required to provide assent, Appendix 10.6.

Potential Risks

According to the guidance provided by the Office of Human Research Protections (US Department of HHS.), participation in this study involves a research activity that presents no more than minimal risk for the volunteers (US Department of HHS.) All participants were subjected to echocardiography, together with routine height and weight measurement. Transthoracic echocardiogram is a non-invasive and non-painful procedure.

Known Potential Benefits

Participants in this study were investigated by the study echocardiographer. The potential benefits of participating in the study are that children with previously undiagnosed RHD would be referred to a tertiary center for prophylaxis, potentially preventing them from progression to life-threatening RHD. Participants were not paid for participating in the study.

2.4.5 Summary of Study Evaluations

The research assistant verified parental / legal guardian consent permission, and in addition, obtained assent from participants 8 years of age or older. Each participant was assigned a unique number and cross-referenced in the enrolment log register. All evaluations were conducted in the purpose-built mobile echo surveillance unit parked on school grounds. A standard CRF was used (See Appendix 10.7). Enrolled participants were interviewed by the study researcher to determine general health, past history of rheumatic fever, frequency of sore throats, and symptoms of ARF at the time of the study examination. Heights and weights were also recorded at the time of the interview.

2.4.5.1 Throat Swab Collection

A selection of participants, regardless of physical findings were swabbed for microbiological investigation for GAS pharyngeal carriage. The throat swab procedure and processing was identical to that of the clinic-based study and is described in Section 2.3.5.

2.4.5.2 emm Typing

GAS isolates were submitted to the Division of Microbiology for *emm* typing which involves a number of steps. Briefly, following DNA extraction, PCR amplification is performed using recommended primers. The PCR products are separated and visualized using agarose gel electrophoresis and purified. After sequencing, a comparison is made with *emm* sequences in the existing databases housed at the Centres for Disease Control and Prevention (CDC), at which time the sequence is assigned its corresponding sequence number. More detail is provided in Appendix 10.8.

2.4.5.3 Echocardiography

All echocardiographic studies were performed in the left lateral decubitus position, initially using a Vivid i machine (GE Ultrasound, South Africa), equipped with a 3S probe and tissue Doppler technology. Other machines used included the Philips® CX50 with an S5-1 phased-array transducer probe and the Sonosite® M-Turbo with the P10 probe. Simultaneous electrocardiography was used to correlate timing of electrical events with mechanical events. No sedation was used.

Echocardiography was performed by experienced cardiac ultrasonographers who had received extensive training in adult and paediatric echocardiography at teaching hospitals in Cape Town. Standard approaches to trans-thoracic echocardiography were adopted. All M-mode measurements were taken and recorded at least twice, while the Doppler velocity of greatest value and intensity was recorded. Measures found to be abnormal in these reference ranges were compared to standard values for body surface area.

Echocardiograms were reviewed by a cardiologist blinded to the original echocardiographer's preliminary diagnosis. Where there was a difference of opinion or any uncertainty, the opinion of a second cardiologist was sought, and decisions were based on consensus between all three individuals. Initial analysis of our echo data was done using the NIH/World Health Organization expert consultation criteria for diagnosis of RHD published in 2006 (Carapetis JP et al., 2006).

The criteria initially used to report school screening echocardiograms were based on a combination of clinical and echocardiographic findings. Study patients were classified as definite, probable, or possible RHD, congenital cardiac disease, or normal. This approach however, implied that a fully trained cardiologist needed to be present in order to auscultate each patient. We developed and used a new set of criteria which focused on the echocardiograph as a screening tool without the concomitant use of clinical examination in the screening phase. (Figure 2.4) (Zuhlke and Mayosi, 2009).

The most recent development however, has been the publishing of the evidence-based World Heart Federation guidelines for echocardiographic diagnosis of RHD, but these only became available after completing this study.

2.4.6 Data Handling And Record Keeping

Demographic data, collected at the time of screening by the field site coordinator, were entered directly into a customised database (Epi Info™ software package). For the echocardiographic examination, detailed CRFs were used to categorize the findings from

Definite RHD
Significant mitral stenosis (mean gradient: >4mmHg)
Significant structural and/or functional changes involving both mitral and aortic valves, i.e., multiple valve disease
Probable RHD
Significant structural and functional changes involving either mitral or aortic valves, i.e., single valve disease
Possible RHD
Isolated structural OR functional changes involving either mitral or aortic valve

Definitions	
Significant structural changes: Thickness of mitral and aortic leaflets greater than 4mm	**Significant functional changes:** ***Significant mitral regurgitation:*** defined as a mitral regurgitant jet at least 1 cm from the coaptation point of the valve leaflets, seen in two planes, high velocity (mosaic pattern) and persisting throughout systole. Additional changes that may be present include multiple regurgitant jets and/or a posterolaterally-directed jet
Increased echogenicity of submitral structures	
Rheumatic nodules giving a beaded appearance	
Prolapse of mitral, aortic or tricuspid valves	
Reduced mobility of leaflets	***Significant aortic regurgitation:*** defined as an aortic regurgitant jet at least 1 cm from the coaptation point of the valve leaflets, of high velocity (mosaic pattern) and seen in two planes
Chordal tears	
Elbow or dog leg deformity of the anterior mitral valve leaflet.	
Fixed or markedly restricted motion of the posterior mitral leaflet	

Figure 2.4. Echocardiographic criteria used in the Vanguard Screening study

all participants as normal, probable RHD or definite RHD as per the echocardiographic criteria according to the Zühlke criteria (Zuhlke and Mayosi, 2009). These findings are captured into the database at the Data Entry Centre in the offices of the Mayosi Research Group. After the confirmatory examination by the cardiologist, those participants with definite or borderline RHD are referred to their primary care clinic with appropriate documentation for follow-up and secondary antibiotic prophylaxis. Data were reviewed

on a regular basis and queries were referred to the field site coordinator for comment. All CRFs and related query documents and data quality reports are filed in a fire-proof lockable cabinet.

2.4.7 Statistical Considerations

2.4.7.1 Outcome Measures

- The proportion of school-going children with echocardiographic features of RHD
- The age-, gender- and suburb-specific background rates of GAS pharyngeal carriage in school-going children living in the Vanguard area of Cape Town

2.4.7.2 Sample Size Considerations

GAS pharyngeal carriage

The sample size for this nested study was based on published studies in similar South African communities (McLaren et al., 1975, Ransome et al., 1983) allowing for estimation of at least 5% difference of GAS carriage across the two communities within a precision of 5%; in addition, we assumed a 5% non-response rate. Therefore, at least 451 participants needed to be examined per community.

Prevalence of RHD

A necessary minimum total sample size of 2875 subjects was calculated, anticipating a frequency of 3% of RHD. This sample size also assumes a rejection rate of 5%. With this sample, we would be able to estimate the prevalence of RHD within 0.7 percentage points of precision with 95% confidence. A 1.2 design effect (i.e. an increase of 20% on the

original sample size) to account for clustering in the sampling design is assumed between the participants coming from the same classroom. The 3% was taken from a recent screening carried out among school children in Mozambique (Marijon et al., 2007). Our total school population was 16,771 pupils.

In South Africa, a previous prevalence survey estimated the prevalence of RHD in school children from poor households as 10-15 per 1,000 (Maharaj et al., 1987). Table 2.3 shows the total number of participants in each municipality and the expected sample distribution among the two juxtaposed peri-urban townships, Langa and Bonteheuwel, forming the Vanguard area, reaching the total sample size of 2875.

Table 2.3. Expected sample size distribution by region

Municipality	Total (%)	Sample Required
Langa	8034 (47.9)	1377
Bonteheuwel	8737 (52.1)	1498
Total	16 771 (100)	2875

2.4.7.3 Analysis Plan

We estimated the prevalence of RHD in Vanguard with the corresponding 95% confidence interval. The estimate of the standard error for this estimate accounted for clustering effects and was considered in the confidence interval computation. Similar estimates are provided by region, gender, and selected age groups. We also describe the background characteristics and other clinical features of RHD cases collected in this study.

3 HOST DETERMINANTS: STUDY ONE

SUSCEPTIBILITY TO DEVELOPING RHEUMATIC HEART DISEASE: A SYSTEMATIC REVIEW OF THE GENETIC EVIDENCE FROM TWIN STUDIES

The data presented in this section have been published in the peer-reviewed article:
Engel, ME; Stander, R; Vogel, J; Adeyemo, AA; Mayosi, BM. Genetic susceptibility to acute rheumatic fever: a systematic review and meta-analysis of twin studies. PLoS One. 2011;6(9):e25326.

3.1 INTRODUCTION

The role of host factors is discussed in the literature review (Section 1.3). As mentioned, while much is known about the social factors and the microbial agent that predispose to ARF, little progress has been made in elucidating genetic susceptibility factors that are reproducible in different populations (Bryant et al., 2009).

Several twin and family aggregation studies have suggested a genetic effect, but they have not provided a quantitative estimate of the magnitude of the genetic contribution in ARF and RHD. Furthermore, the molecular genetic studies of human leukocyte antigen (HLA) and non-HLA factors have been characterized by small studies with inconsistent and conflicting findings (Bryant et al., 2009). A quantitative assessment of the genetic effect in twin or adoption studies, which provide a reliable estimate of genetic effect in familial conditions, will provide guidance on the desirability of embarking upon the expensive

'global genome analysis' studies of the condition that have been recommended recently (Bryant et al., 2009) Higher concordance rates between monozygotic twins compared with dizygotic twins indicate a greater role for genetic factors in the development of a disease given that monozygotic twins are genetically identical, versus dizygotic twins who, on average, share 50% of their genes, Conversely, concordance of a similar magnitude in both monozygotic and dizygotic twin pairs suggests the involvement of factors not pertaining to genes (Ahlbom et al., 1997). It would thus be prudent to invest scarce resources on genetic studies of conditions with good evidence of genetic determination particularly in resource-poor countries where ARF is prevalent.

We have conducted a systematic review and meta-analysis of the concordance rate and heritability of ARF in monozygotic and dizygotic twin pairs as reported in observational studies. The primary aim of this study in the thesis was to determine the extent to which the variation of the disease between monozygotic and dizygotic twin pairs is due to genetic effects**.**

3.2 METHODS

The systematic review process was based on the guidelines set out in the Cochrane Handbook for Systematic Reviews (Higgins et al., 2009) and the methods of the Human Genome Epidemiology Network in this review to summarise all studies reporting on occurrence of ARF in monozygotic and dizygotic twins (Little and Higgins, 2006). Independent reviewers assisting with study selection and data extraction is a pre-requisite to eliminate bias; two research assistants (RS, and JV) thus participated in the review

process which was governed by a review protocol. The PRISMA Statement for reporting systematic reviews was used as a guide in writing up this review (Moher et al., 2009).

3.2.1 Data Sources and Search Strategy

Using the terms ((RHEUMATIC FEVER OR RHEUMATIC HEART) AND (FAMIL* OR TWIN OR ADOPTION)), we searched PubMed/MEDLINE, EMBASE, Thomson Reuters ISI Web of Science and Google Scholar for all reports of original research from the inception date of each database to 31 January 2011, without any language restriction. Predefined criteria were used to identify studies examining the concordance for ARF and RHD in monozygotic versus dizygotic twins. The literature search was performed independently by the three reviewers (MEE, RS, and JV). In addition, an independent search specialist conducted an EMBASE search. This process was complemented by reviewing the reference list of all articles identified, and by scanning abstracts from conference proceedings. The search strategy for Medline and EMBASE appears in Appendix 10.9

3.2.2 Inclusion Criteria

We included reports that met the following criteria:

1. Twin studies reporting on the concordance for ARF and/or RHD in monozygotic versus dizygotic twins (i.e., comparative group used);
2. Use of accepted diagnostic criteria for ARF;

3. Clear indication of how zygosity was established; and
4. Diagnostic assessment of relatives preferably performed with investigators blind to the affection status of the proband.

The first three criteria were an absolute requirement for inclusion of a report in the study. Three observers (MEE, RS, and JV) independently evaluated the titles and abstracts of search outputs, and thereafter compiled a list of articles deemed to be potentially relevant. Full-text articles were subsequently retrieved and evaluated against the inclusion criteria.

3.2.3 Validity Assessment of Included Studies

We assessed studies in terms of case definition and determination of twin zygosity wherever recorded by the authors.

3.2.4 Data Abstraction

From each study, reviewers independently recorded the year of publication, origin and demographic details of participants, matching procedures for zygosity, diagnostic criteria for ARF and RHD, and information on disease. The supervisor served as arbitrator where necessary.

3.2.5 Quantitative Methods

Concordance or twin similarity was assessed by calculation of two concordance rates: the pairwise concordance rate and the probandwise concordance rate. The pairwise concordance rate is given by the formula:

$$\frac{\text{Number of pairs where both twins are affected}}{\text{Total number of twins.}}$$

The probandwise concordance rate is given by the formula:

$$\frac{\text{Number of probands whose co-twins are affected}}{\text{Total number of probands.}}$$

The advantage of the probandwise concordance rate is that it is independent of ascertainment. We also calculated aggregate pooled probandwise concordance by combining raw data from each of the studies. Heritability was estimated by estimating the variance components using structural equation modeling techniques as implemented in the MX software package (*http://www.vcu.edu/mx/*). We considered an "ACE" variance components (VC) model incorporating parameters for additive genetic (A), common (shared) environmental (C), and individual specific (unshared) environmental (E) components of the total variance (V). The A, C, and E parameters were estimated by maximum likelihood, under a "liability threshold" model. Heritability (h^2) is the proportion

of the total variance that is attributable to the genetic variance; i.e. h^2=A/V. Odds ratios from separate studies were combined by random-effects meta-analysis according to the Mantel-Haenszel method to evaluate association between zygosity status and concordance. Heterogeneity between studies was evaluated with the χ^2 Q statistic, which was considered significant for $p < 0.1$. STATA software version 11 (STATA Corporation, College Station, Texas, USA) was used to perform the meta-analysis and produce the forest plots using the '*metan*' routine, which automatically adds 0·5 to all zero-containing cells of the 2×2 table before analysis (Sterne JAC et al., 2001).

3.3 RESULTS

3.3.1 Flow of included studies

The electronic literature search yielded 685 articles for consideration (Figure 3.1). Titles and abstracts were reviewed and together with the articles identified by hand searching, twenty-three publications were selected for possible inclusion; however, three non-English language papers were excluded due to unavailability of the full text of the articles from the authors and library archives (Strusberg et al., 1968, Uribarri et al., 1965, Zajicek and Gajova, 1966) [4,5,6]. Of the remaining 20 articles, a further 14 were excluded for the following reasons: no twins reported (n=10) (Benevolenskaia et al., 1973, Benevolenskaia and Miakotkin, 1980, Bobylev and Men'shova, 1972, Giannini and Romani, 1967, Paul, 1941, Paul and Salinger, 1931, Prasad, 1967, Quinn and Federspiel, 1967, Spagnuolo and Taranta, 1968), having rheumatoid arthritis as the outcome (n=1) (Dixon, 1969)[17], or lack of a comparison group (n=2) (Denbow et al., 1999, Perry, 1940). Finally, one study

did not distinguish between types of twins (Honeyman and Davis, 1971) (Table 3.1). We did not discover any unpublished studies nor did we find any twin studies of RHD that met the inclusion criteria.

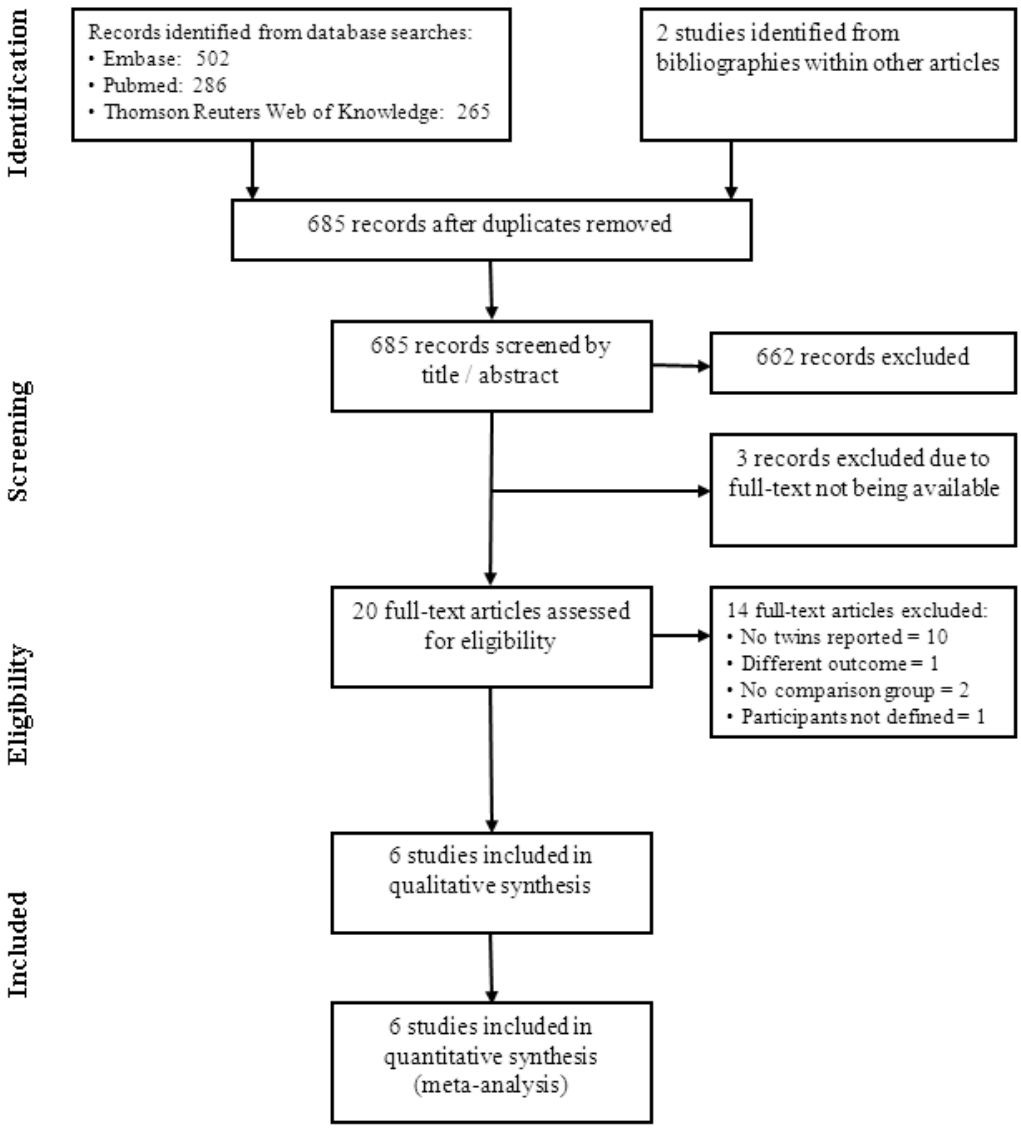


Figure 3.1 Process of arriving at final set of included studies

Table 3.1: Studies that were excluded from the systematic review

Author	Year	Reason for exclusion
Benevolenskaia	1973	No distinction between MZ and DZ twins
Benevolenskaia	1980	No twins reported in sample
Bobylev	1972	No distinction between MZ and DZ twins
Denbow	1999	No comparison group
Dixon	1969	Different Diagnosis (Rheumatoid Arthritis)
Giannini	1967	No twins reported in sample
Honeyman	1971	No distinction between MZ and DZ twins
Horne	2004	No twins reported in sample
Paul(a)	1931	No twins reported in sample
Paul(b)	1931	No twins reported in sample
Perry	1940	Case study, no comparison group
Prasad	1969	No twins reported in sample
Quinn	1967	No twins reported in sample
Spagnuolo	1968	No twins reported in sample
Not available		
Strusberg	1968	Spanish
Uribarri	1965	Spanish
Zajicek	1966	Czech

MZ, monozygotic; DZ, dizygotic

3.3.2 Characteristics of Included Twin Data Sets

Six studies published between 1933 and 1964 and contributing a total of 435 twin pairs were included in the analysis, Table 3.2 (Kauffman and Schreerer, 1938, Reed, 1964, Stevenson and Cheeseman, 1953, Irvine-Jones, 1933, Wilson and Schweitzer, 1937, Taranta et al., 1959). Two of the included datasets (Kauffman and Schreerer, 1938, Reed, 1964) were retrieved from Perry (Perry, 1940) and Honeyman (Honeyman and Davis, 1971) respectively, while another was reported as a conference abstract (Taranta et al., 1959). Where indicated by the authors, studies were conducted in North America (Irvine-Jones, 1933, Wilson and Schweitzer, 1937) and Ireland (Stevenson and Cheeseman, 1953). One study (Taranta et al., 1959) specifically focused on twins, while in the others, twin pairs formed part of larger cohorts. Wilson used previous records of children attending a pediatric cardiac clinic to obtain twin samples (Wilson and Schweitzer, 1937).

In all six studies the outcome measured was the concordance of acute rheumatic fever in monozygotic and dizygotic twin pairs. Defining characteristics of the studies are presented in Table 3.3. Only two studies provided information on how zygosity was determined (Stevenson and Cheeseman, 1953, Taranta et al., 1959); methods included evaluation of blood groups, similarity of dermatoglyphics, hair and eye colour. Information on age and gender was incomplete or not specified: one study comprised only females in the MZ group while the DZ twin sets included both females and mixed gender (Stevenson and Cheeseman, 1953). Another study consisted of mixed and same sex twin sets in a 3:5 ratio (Taranta et al., 1959). Where indicated, the diagnosis of acute rheumatic fever was made

Table 3.2: Observational studies of zygosity and concordance for rheumatic fever

Study ID	No of twin pairs	Type of zygosity	Occurrence of acute rheumatic fever	
			Concordance	*Disconcordance*
Irvine-Jones 1933	7	MZ	2	0
		DZ	0	5
Wilson 1937	6	MZ	2	0
		DZ	2	2
Kaufmann 1938	72	MZ	5	22
		DZ	1	44
Stevenson 1953	10	MZ	1	0
		DZ	0	9
Taranta 1959	56	MZ	3	13
		DZ	1	39
Reed 1964	284	MZ	36	91
		DZ	11	146
Totals	435	MZ	49	126
		DZ	15	245

No, number; MZ, monozygotic; DZ, dizygotic

Table 3.3: Possible sources of bias: defining characteristics in individual studies

Study ID	ARF case definition	Gender of twin pairs	Zygosity Determination
Irvine-Jones 1933	ARF, chorea, joint pain, fever absent, mitral stenosis	nd	nd
Wilson 1937	New York TB and Heart Association	nd	nd
Kaufmann 1938	nd	nd	nd
Stevenson 1953	ARF, chorea, mitral stenosis, death from RHD, rheumatic conditions with mitral involvement	MZ:F; DZ: 6=same sex, 3=mixed	nd
Taranta 1959	Jones' criteria	MZ:nd; DZ: 23=same sex, 17=mixed	blood group, hair and eye-colour, similarity of dermatoglyphics,
Reed 1964	nd	nd	nd

ARF, acute rheumatic fever; nd, no data; RHD, rheumatic heart disease; MZ, monozygotic; DZ, dizygotic; F, female; M, male

either using the criteria of the Heart Committee of New York Tuberculosis and Health Association, Inc or the Jones Criteria (Stevenson and Cheeseman, 1953, Taranta et al., 1959, Wilson and Schweitzer, 1937, Irvine-Jones, 1933).

3.3.3 Relationship between Zygosity and Concordance Rates of ARF

Probandwise concordance rates ranged from 31% to 100% for monozygotic twins, and from 0% to 67% in dizygotic twins in individual studies. The pooled probandwise concordance rate was 44% for monozygotic twins and 12% for dizygotic pairs. Random-effects meta-analysis confirmed the strong association between zygosity and concordance for acute rheumatic fever in twins (OR, 6.39; 95% CI, 3.39 to 12.06; P<0.001) (Figure 3.2).

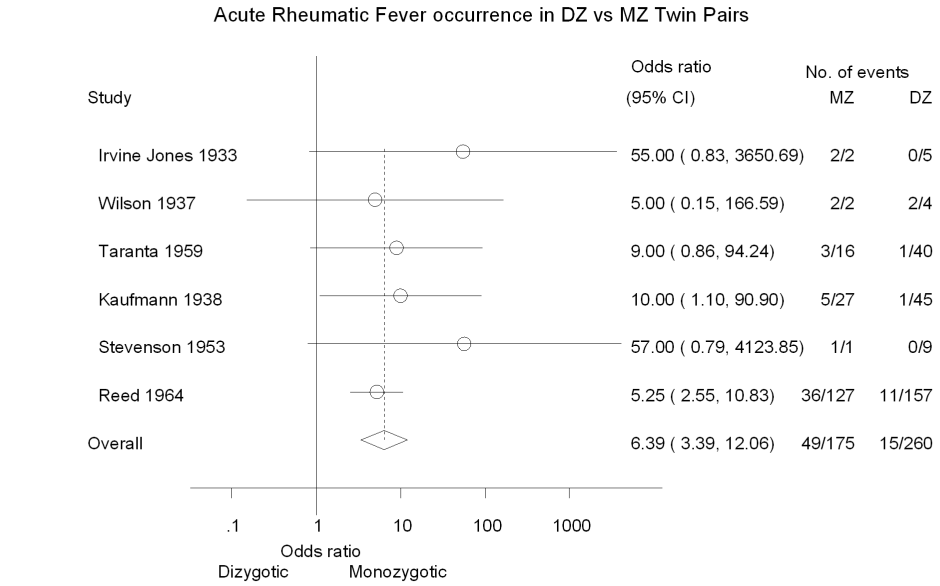


Figure 3.2. Odds ratio of concordance for acute rheumatic fever according to type of zygosity
Horizontal lines, 95% confidence interval; CI, Confidence interval; MZ, monozygotic; DZ, dizygotic

If the two studies with no events among dizygotic twins are excluded, the pooled OR = 5.78 (95% CI, 3.02 to 11.05; P<0.001). No statistical heterogeneity was present between studies (χ^2 2=2.56; P=0.768). Heritability estimated by fitting VC models is shown in Table 3.4. Study-specific heritability estimates of acute rheumatic fever from the three larger studies varied from 0.54 to 0.73. The heritability across all the studies was 0.603 (95% confidence interval 0.413, 0.805).

The comprehensive literature search revealed no twin studies of concordance of rheumatic heart disease.

3.4 DISCUSSION

To the best of our knowledge, this meta-analysis of 175 monozygotic and 260 dizygotic twin pairs represents the largest quantitative assessment of the heritability of acute rheumatic fever to date. This study shows that the risk of acute rheumatic fever in a monozygotic twin with a history of acute rheumatic fever in the co-twin is increased by more than six times compared to that of dizygotic twins. The heritability estimate was 60%, which confirms the importance of genetic factors in acute rheumatic fever. These findings provide a strong justification for embarking on whole genome mapping of genetic susceptibility variants for acute rheumatic fever in large appropriately designed cohorts (Bryant et al., 2009).

Twin studies provide the appropriate framework for assessing the extent to which the familial occurrence of acute rheumatic fever is due to genetic and environmental factors. Monozygotic and dizygotic twins generally share similar postnatal environments; hence

Table 3.4: Results of fitting the 'ACE' threshold model to estimate genetic and environmental components of

Study ID	No of twin pairs	Additive genetic h^2	Common environment c^2	Unique environment e^2
Kaufmann	72	0.728 (0.134-0.856)	0.000 (0.000-0.503)	0.272 (0.144-0.454)
Taranta 1959(Taranta	56	0.714 (0.017-0.887)	0.018 (0.000-0.565)	0.268 (0.114-0.513)
Reed 1964(Reed,	284	0.540 (0.315-0.781)	0.275 (0.051-0.474)	0.185 (0.135-0.246)
All studies**	435	0.603 (0.413-0.805)	0.209 (0.023-0.378)	0.188 (0.144-0.239)

*'ACE', **A**dditive genetic factors, **C**ommon environment, and unique **E**nvironment; No, number*

**Limited to studies with sufficient numbers (> 50) of twin pairs*

***Includes subjects from all six studies*

phenotypic similarity in monozygotic twins that is greater than that of dizygotic twins is due to their greater genetic similarity (Phillips, 1993). To compare the different studies, we used the probandwise concordance rate to derive the proportion of affected co-twins for an affected proband in a particular study; the probandwise concordance rate has the advantage of providing the opportunity to compare studies irrespective of their ascertainment method, and provide an overall estimate of twin concordance rates for a particular condition. The important role which genetic susceptibility studies can play in informing the prioritization of research resources is illustrated in the unraveling of genes involved in breast cancer, where monozygotic twin pairs carry a risk of almost three times of developing the disease compared with dizygotic twins or first-degree relatives (Mack et al., 2002).

Acute rheumatic fever is a complication of untreated streptococcal pharyngitis for which only affects 3-6% of the general population is at risk. It is not possible at present to predict the individuals who are at risk of developing acute rheumatic fever following an episode of streptococcal pharyngitis. The identification of genetic variants that reliably predict risk of development of acute rheumatic fever may be used to identify individuals who may benefit from prophylaxis with penicillin or vaccination against invasive streptococcal infection. This study suggests that genetic factors may have a high predictive power for the development of acute rheumatic fever, and as such may be of clinical utility in predicting disease risk. Therefore, the identification of all genetic susceptibility factors for acute rheumatic fever through whole genome analysis may lead to the development of a useful predictive genetic risk score for the disease.

This review has several limitations. First, there is the possibility of misclassification of

the phenotype given that we used the clinical definition of acute rheumatic fever provided by the authors. Three of the studies were published before the original Jones criteria for the diagnosis of acute rheumatic fever were formulated in 1944, and relied on the clinical judgment of the investigators. Thus, given that the criteria for the diagnosis of acute rheumatic fever have evolved over the years, the generalizability of our findings to the present may be limited. Secondly, there was incomplete information on the age and gender of the twins included in this review. It is therefore not possible to assess the effects of age of onset or gender on genetic susceptibility to acute rheumatic fever in this work. Thirdly, there is a lack of data on the methods of determining zygosity status in four of the studies, which may cast doubt on the reliability of the findings. Although no twin studies were found for RHD, it is likely that these findings apply to cases of RHD nonetheless, which is a consequence of repeated attacks of ARF. Finally, it must be acknowledged that the results of twin studies cannot automatically be generalized beyond the population in which they were derived, and heritability is specific to a particular population in a particular environment. Furthermore, these studies were carried out over 45 years ago mainly in industrialized countries where endemic rheumatic fever has since been eradicated through improved living conditions and the availability of penicillin; today acute rheumatic fever is largely a disease of poor communities in developing countries. No studies of familial aggregation of the disease have been done within the developing country setting, which possibly reflects the relative neglect of this disease by the research community (Watkins et al., 2009) It is interesting, however, to note that the proportion of people at risk of development of acute rheumatic fever following untreated streptococcal pharyngitis remains the same in all ethnic groups of the world (Bryant et al., 2009). It is likely therefore, that there are no major differences in the magnitude of genetic determination of acute rheumatic fever in

human populations. It follows therefore, that the apparent ethnic variation between racial groups are more likely to be due to confounding by socio-economic factors, especially given that socioeconomic factors are difficult to measure completely (Kaufman et al., 1997)

In summary, this meta-analysis of 435 twin pairs with acute rheumatic fever shows that the monozygotic twin concordance substantially exceeds the dizygotic twin concordance, and thus implicates genetic factors as playing a significant role in the etiology of the condition. These results should provide renewed impetus to the establishment of large-scale studies to unravel the genetic architecture of acute rheumatic fever and rheumatic heart disease. Finding reliable and reproducible genetic susceptibility variants for acute rheumatic fever will not only assist in elucidating the pathophysiological mechanisms of the disease, but also assist in identifying individuals at risk of the condition in affected communities.

4 PHARYNGEAL CARRIAGE OF THE AGENT: STUDY TWO

CARRIAGE RATES AND GENOTYPIC CHARACTERISTICS OF GROUP A STREPTOCOCCI IN ASYMPTOMATIC SCHOOL CHILDREN OF THE VANGUARD COMMUNITIES OF CAPE TOWN, SOUTH AFRICA

4.1 INTRODUCTION

The epidemiology of pharyngeal carriage of GAS has been reviewed in the *Background to the Thesis*, Section 1.5.1.2. Data on GAS carriage from African countries remain scant with a few studies reporting a prevalence of about 10% in pharyngeal isolates from healthy participants (Abdissa et al., 2011, Mzoughi et al., 2004, WHO, 2004). In South Africa, four studies conducted in various race groups in the 1970s reported carriage rates varying from 3.6% to over 20% in urban settings where overcrowding, amongst other factors, was identified as a possible predisposing factor to high carriage rates (McLaren et al., 1975, Ransome et al., 1983, Van Staden et al., 1982, Bundred, 1986).

Determining the background prevalence of GAS carriage could aid in identifying risk factors associated with carriage in school-aged children and influence the planning and evaluation of management programmes in the screening of pharyngeal carriers (Lloyd et al., 2006). Also, knowledge of the pretest probability influences assessment of the posttest probability of GAS pharyngitis, so as to minimise unnecessary diagnostic testing in children (Shaikh et al., 2010). Finally, in the light of recent advances towards a streptococcal vaccine (Dale et al., 2011) and given that asymptomatic carriers have been shown to maintain the carrier streptococcal strain when progressing to active disease (Martin et al.,

2004), identification of the DNA sequencing pattern of the 5' hypervariable region of the cell-surface M-protein (so called *emm* typing (Beall et al., 1996)) may be able to inform vaccine development and later, for assessing the impact of vaccination and monitoring serotype changes.

This study was conducted in order to determine GAS carriage rates among asymptomatic school children in the two peri-urban school districts of Bonteheuwel and Langa, Cape Town, South Africa. In particular, we sought to determine the extent to which rheumatogenic strains used for candidate vaccine development were being carried in asymptomatic children. Also, we characterised the *emm* types of the isolates in order to establish whether some strains were more prevalent than others as well as to contribute to the knowledge base informing the development of appropriate GAS vaccines.

4.2 MATERIALS AND METHODS

4.2.1 Study Design and Setting

This cross-sectional study formed part of a larger school-based study on the prevalence of RHD amongst healthy school children in the Vanguard area of Cape Town (See Section 2.1). The area, situated about 14 km from the Cape Town CBD, covers approximately 6.3 km^2 and comprises two lower socio-economic communities of largely black African and mixed ancestry. Approximately 20% of the population is 5 – 15 years of age (Statistics South Africa, 2003). Further detail as regards some of the indicators of socio-economic status and other baseline characteristics are described in detail in Section 2.1 of this thesis.

Recruitment and Sample Size

The sample comprised asymptomatic children between the ages of 5 and 21 years whose parents provided informed consent for participation in the study. The sample size was based on published studies in similar South African communities (McLaren et al., 1975, Ransome et al., 1983) allowing for estimation of at least 5% difference of GAS carriage across the two communities within a precision of 5%; additionally we assumed a 5% non-response rate. Therefore, at least 451 participants per community were needed to be examined over the three-year (school) period from February 2009 to November 2011. A purpose-built database on the Epi Info (version 3.5.2) platform was used to manage the data.

4.2.2 Study Procedure

The throat swab sample was taken by swabbing the tonsillar and posterior pharyngeal areas; samples were transferred in transport media for processing by the Microbiology Laboratory of the National Health Laboratory Service located at Groote Schuur Hospital. Briefly, each throat swab was inoculated onto 4% sheep blood agar plates according to the standard protocol, inverted and incubated anaerobically at 35 ^{o}C for 24-48 hours. All cultures of beta-hemolytic colonies were further identified by Gram stain, catalase, and serogrouping, as appropriate.

The *emm* typing procedure was performed according to established protocols (Beall et al., 1996). Briefly, DNA was extracted from single colonies of GAS and subjected to PCR-amplification using primers targeting the N terminal of the *emm* gene. The PCR product was sequenced according to the CDC protocols (CDC Protocol for emm typing available at

http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm) using ABI Prism® BigDyeTM Terminator Cycle Sequencing kit (Applied Biosystems, USA) and analysed using BioEdit v7.0.9 (Ibis Biosciences, USA). Sequences were submitted electronically to the *S. pyogenes emm* sequence database centre at CDC, which assigned the *emm* types and subtypes.

4.2.3 Ethical Approval

The study was performed with the approval of the University of Cape Town Faculty of Health Sciences Research Ethics Committee, and informed consent was obtained in writing from a parent or legal guardian of each participant. In addition, children aged 8 years and older were required to provide assent.

4.2.4 Statistical Analysis

Statistical analyses were performed using STATA version 11.0 (SPSS Inc., Chicago, IL, USA). Comparisons were made using chi-square test or Fisher's exact test. A p-value of <0.05 was considered indicative of a statistically significant difference.

4.3 RESULTS

4.3.1 Enrolment

Over the three school years (2009 – 2011) of our study, 950 healthy learners attending schools within the two communities of our demonstration site were enrolled into our study. The mean and median ages of the participants were 11.4 years and 10 years respectively (range 3-24 years); males constituted 43% of the study participants (Table 4.1).

Table 4.1. **Comparison of some of the characteristics with respect to GAS status, of the asymptomatic school children recruited into the study during February 2009 – November 2011**

	βHS (+) *(%)*		βHS (-) *(%)*	Total
	GAS + *(%)*	**GAS – (%)**		
Age (mean (sd); [range])	11.1 (3.6); [6-21]	11.6 (4.0); [4-19]	11.4 (4.0); [3-24]	-
< 9 years	7 *(3.1)*	8 *(3.57)*	210 *(93.3)*	*225* (23.7)
≥ 9 years	24 *(3.3)*	43 *(5.9)*	658 *(90.8)*	*725 (*76.3)
Gender				
Female	15 *(2.8)*	35 *(6.4)*	493 *(90.8)*	*543* (57.2)
Male	16 *(3.9)*	16 *(3.9)*	375 *(92.2)*	*407 (*42.8)
School District				
Bonteheuwel	8 *(1.6)**	25 *(5.1)*	454 *(93.2)*	*487* (51.3)
Langa	23 *(5.0)*	26 *(5.6)*	414 *(89.4)*	*463* (48.7)
Season				
Winter	18 *(4.1)*	14 *(3.2)*	406 *(92.7)*	438 *(46.1)*
Summer	13 *(2.5)*	37 *(7.2)*	462 *(90.2)*	512 *(53.9)*
Total	**31 *(3.3)***	**51 *(5.4)***	**868 *(91.4)***	**950 *(100)***

βHS, βeta Haemolytic Streptococcus; GAS, group A streptococcus; (+), positive; (-), negative; %, percentage; sd, standard deviation. * P value < 0.05 for association between school district and having a GAS (+) status

4.3.2 β-haemolytic streptococci isolation

β-haemolytic streptococci (βHS) were identified in swabs from 82 of 950 participants (8.6% (95% Confidence Interval [CI]: 6.84 – 10.4 %)). There was no association between βHS isolation and gender (p=0.47), age < 9 years vs age ≥ 9 years (p=0.23) or winter vs summer

seasons (p=0.18). More βHS were isolated from participants in the Langa school district (10.6%) than from those in Bonteheuwel (6.7%), (p=0.037).

4.3.3 Group A Streptococci Isolation

Group A Streptococci (GAS) was isolated from 37.8% of all βHS isolates (31/82) corresponding to a carrier rate of 3.26% (95% CI: 2.13 – 4.39 %) among healthy school-aged learners (Table 4.1). GAS was recovered from almost all ages of learners (Figure 4.1)

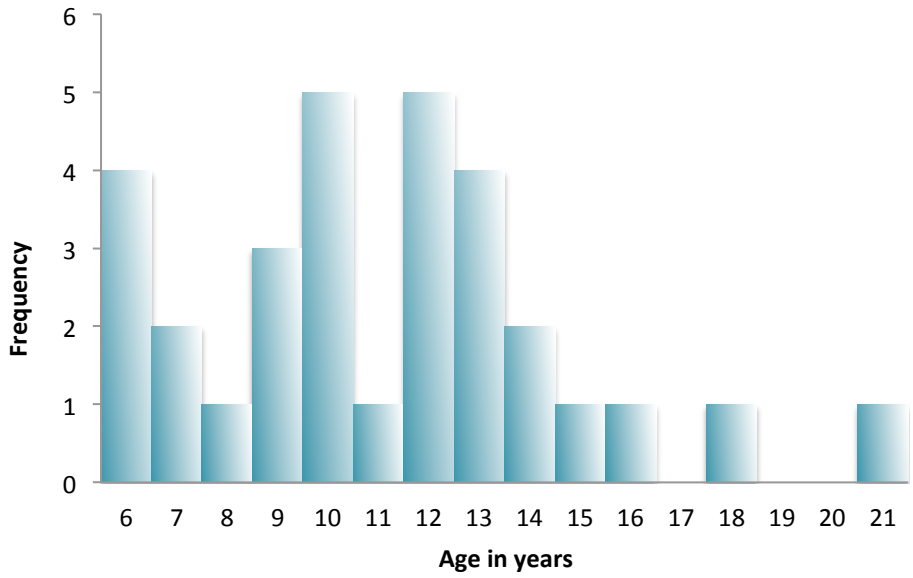


Figure 4.1. Age distribution of GAS in asymptomatic school children

(mean age = 11.09 years (sd ± 3.6 yrs) with no association of GAS status with age (p=0.628). Winter vs summer showed no significant association with GAS recovery as did neither gender. Considering school district, there was a statistically significant difference in

the isolation rates of GAS by school district, with pupils from Langa, having a significantly increased odds of having the organism isolated (O.R 3.129, 95% CI 1.38 – 7.09).

4.3.4 Non-Group A Streptococci Isolation

The carrier rates amongst the non-Group A βHS isolates were 3.5% (33/950) for group C (GCS) and 1.5% (14/950) for group G streptococci (GGS). Other βHS recovered included group F (3 isolates) and group D (1 isolate) (Table 4.2).

Table 4.2. **Seasonal distribution of beta haemolytic streptococci isolates from asymptomatic school children**

Climate	GRP-A	GRP-C	GRP-D	GRP-F	GRP-G	Total
Winter	18	10	0	0	4	**32**
Summer	13	23	1	3	10	**50**
Total	**31**	**33**	**1**	**3**	**14**	**82**

GRP, Group

4.3.5 *emm* Typing of Streptococcal Isolates

We managed to sequence 25/31 of the GAS isolates in the time available for this study. There was a variety of *emm* types represented, and the most common types were emm 4 (12%) and emm 9 (12%) (Figure 4.2). Of the 25 isolates investigated, 14 are represented in the putative GAS 30-valent vaccine (Dale et al., 2011).

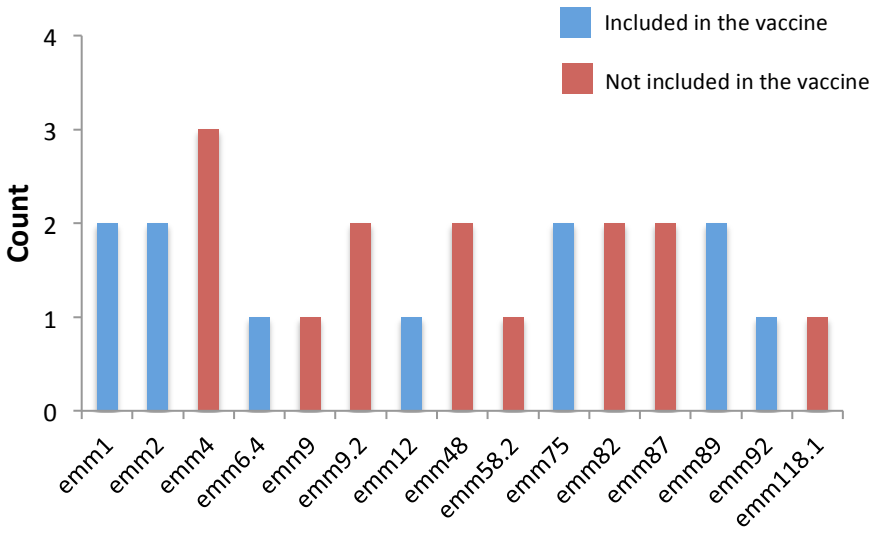


Figure 4.2. Distribution of emm types in GAS isolated from asymptomatic school children

4.4 DISCUSSION

This study investigated GAS carriage in a population of South African school children in order to determine carrier rates, prevalent serotypes and *emm* strains, and their association, if any, with a number of demographic factors. Carriers of GAS may represent a potential source of the acquisition of infections for other children and adults. A longitudinal study over 44 months demonstrated the persistence of carrier strains during repeated episodes of GAS infection in 50% of participants (Martin et al., 2004). Thus, GAS carriage reflects the reservoir of circulating strains, which are relevant to disease such as pharyngitis

Microbiological evidence of βHS carriage was found in 8.6% of pharyngeal swabs taken from 950 healthy school-aged children. The proportion of GAS carriage was observed to be 3.3% among the 950 healthy school-aged children, with no difference in the prevalence rates for male and female school children. Prevalence rates of GAS carriage of ≥ 10% have been reported in school settings in both industrialised and developing countries

(Gunnarsson et al., 1997, Jasir et al., 2000, Kim and Lee, 2004). A meta-analysis comprising 18 studies conducted in clinic and school settings in both developed and developing countries reported a pooled prevalence of GAS carriage of 12% (95% CI: 9%–14%). Our present rate of GAS carriage is below previously reported carriage rates from South Africa; furthermore, we did not observe the seasonal variation between winter and summer isolation rates, which may be related in part, to the climatic differences between Cape Town and the other regions in South Africa where the respective studies were conducted (Ransome et al., 1983).

In our study, carriage of group A streptococci in the Vanguard Community, a community with apparently high rates of rheumatic fever and rheumatic heart disease, is remarkably low (overall3.3%). A similar low carriage rate (3.7%) of GAS was reported in a study from the Northern Territory of Australia (McDonald et al., 2006). GAS carriage was significantly associated with the Langa school district; learners from Langa were found to have more than three times the odds of GAS carriage ($p=0.038$). While both Bonteheuwel and Langa school districts are considered as being of low SES, of the two, Langa rates poorly in a number of key indicators of SES. Thus, the statistically significant higher prevalence observed in Langa may be explained by socioeconomic factors such as overcrowding and lack of suitable housing. This finding corroborates those of earlier studies conducted in South Africa amongst urban Black communities (Van Staden et al., 1982, McLaren et al., 1975), historically known to have resided in overcrowded poverty-stricken conditions.

Rates of carriage for GCS and GGS were 3.5% and 1.5% respectively. The potential pathogenicity of non-GAS organisms is not well understood with there being some

evidence for an association with pharyngitis and other diseases (Turner et al., 1997, Tiemstra and Miranda, 2009, Zaoutis et al., 2004). However, these findings were not confirmed in a study conducted in the South Pacific (Steer et al., 2009).

This is only the second study to document the molecular epidemiology of GAS isolates from asymptomatic carriers in Africa. The 25 isolates recovered from asymptomatic school children in our study represents 15 *emm* types; although *emm* 4 accounted for the largest proportion, there was no predominant subtype. Our isolates have only seven *emm* types in common with those reported from asymptomatic carriers in Ethiopia (Abdissa et al., 2006). Interestingly, our most abundant *emm* type, *emm* 4, was absent from their cohort . We considered our *emm* types in light of the putative vaccine under development by Dale and colleagues; only forty-six per cent of our *emm* types are listed among those in the 30-valent vaccine (Dale et al., 2011).

This study screened sufficient participants in terms of the size required for this study. However, there are a number of limitations: while we are certain that we only sampled healthy children, we nevertheless had to rely on participants' self-reporting as regards recent pharyngitis and antibiotic history given that we did not serologically confirm the absence of a recent episode of GAS. We were also limited by the short summer school terms due to holidays, and thus seasonal variation across all four seasons could not be adequately captured in terms of when swabs were taken. Given that only 25 isolates were sequenced, it is difficult to draw firm conclusions regarding dominant *emm* types. No serology testing was undertaken, thus reducing the discriminatory power to distinguish between cases and carriers.

In conclusion, we have shown that among asymptomatic school children from Cape Town, GAS carriage was higher in Langa than Bonteheuwel. Furthermore, there is a diversity in *emm* type distribution of which more than 50% are not included in the putative 30-valent vaccine. Further research needs to be conducted to elucidate the possible role of GAS carrier strains in episodes of acute GAS pharyngitis.

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5 EPIDEMIOLOGY OF GAS PHARYNGTIS: STUDY THREE

LONGITUDINAL STUDY OF GAS PHARYNGITIS IN THE VANGUARD COMMUNITY DEMONSTRATION SITE: INCIDENCE AND PREVALENCE IN CHILDREN AGED 3 TO 15 YEARS

5.1 INTRODUCTION

As discussed in Section 1.4, GAS pharyngitis in childhood has been shown to be associated with the subsequent development of ARF in up to 3% of patients, following an autoimmune response in untreated patients (Rammelkamp et al., 1952). Furthermore, subsequent GAS infections increase the risk of having a recurrent episode of ARF to as high as 75% (Denny, 1987).

The incidence of GAS pharyngitis is estimated to be 616 million cases per year amongst all ages across the world based on a systematic review of population-based data using United Nations population data as the denominator; in more developed countries, approximately 15% of school-aged children will suffer an episode of GAS pharyngitis each year, whereas in less-resourced countries, the incidence may be more than five times greater (Carapetis et al., 2005b). Previous studies have reported varying incidence rates of acute sore throat ranging from 8,3 to 705 cases per 100 child-years and GAS swab-positive pharyngitis from 3,9 to 95 cases per 100 child-years (see Section 1.5). Only two studies have reported on the prevalence of GAS in participants with pharyngitis in South Africa. Throat swab culture returned a positive culture in 33% and 42% in each study

respectively, conducted in Pretoria (van Zyl et al., 1981) and Bloemfontein (Olivier and de Graad, 1978) more than 30 years ago.

An understanding of the incidence of GAS pharyngitis in children within a local context is an important component of any ARF and RHD control programme (World Health Organization., 2004). Addressing GAS pharyngitis through appropriate primary prevention measures (as discussed in Section 1.6) and treating all symptomatic GAS sore throats with a course of oral or parenteral penicillin (Robertson et al., 2005a) presents the opportunity for primary intervention of RHD (Karthikeyan and Mayosi, 2009), thereby reducing the economic and major public health consequences associated with disease burden (Pfoh et al., 2008, Danchin et al., 2004).

The primary aim of this study was to determine the incidence and prevalence of GAS pharyngitis among children of the Vanguard community of Cape Town over a four-year period.

5.2 PATIENTS AND METHODS

The methods are described previously in detail in Section 2.3 'Rationalé and Design of the studies".

5.2.1 Study Design

We conducted a 4-year prospective, clinic-based study from May 2008 – April 2012. A research nurse identified children with symptoms of sore throat among attendees of the three community-based clinics.

5.2.2 Setting and Participant Recruitment

Participants aged 5 – 15 years were enrolled, following informed consent, according to a standard protocol from one of the three clinics serving the Vanguard community of Cape Town. Children >7 years also provided assent to their participation in the study. The two communities of Bonteheuwel and Langa within Vanguard differ significantly with respect to a number of socio-economic status indicators (Section 2.1). Each participant was designated a unique identifying code. Prior to obtaining the throat swab specimen, the research nurse performed a physical examination of the pharynx and recorded, in addition to the demographic characteristics of participants, recent antibiotic usage, which was verified by folder review; patients who had received antibiotics in the preceding 30 days were not enrolled in the study. The symptoms and signs were documented in a database. A repeated episode of sore throat was treated as a new case.

5.2.3 Treatment of Pharyngitis

The research study did not interfere with the routine standard of care at the respective institutions. Thus, patients were treated according to the clinic protocols for sore throat management. A copy of the laboratory culture results of all study participants was placed in the clinic folder.

5.2.4 Laboratory Methods

Specimen Collection, Transport and Handling are outlined in Section 2.3.6. Briefly, all throat swabs were placed in individual sealed specimens and delivered to the microbiology laboratory as soon as possible following collection (usually by midday). A batch flow form was used to track specimens and accompanying CRFs. On arrival at the

laboratory, specimens were plated onto 4% sheep blood agar plates, incubated anaerobically at 35°C, and read within 18 to 24 hours. Patients were characterized as GAS positive if beta-haemolytic colonies were confirmed as *Streptococcus pyogenes* by Gram stain. A single colony was picked off with a sterile wire loop, and sub-cultured for purity. Pure colonies of beta-haemolytic streptococci identified as group A, C or G were removed from the plates in a sterile fashion and placed in trypticase soy broth with glycerol for storage.

5.2.5 Statistical Analyses and Denominator Figures for Incidence Calculations

Statistical considerations are discussed in Section 2.3.8; about 420 cultures were expected per annum at the clinics collectively. I compared categorical data according to demographic and clinical profiles using χ^2 or Fisher's exact test (for small numbers) with calculation of odds ratios (OR) and 95% confidence intervals (CI) as appropriate. To facilitate comparisons, I generated new variables: ***AgeCat***: for age categories of < 9 vs ≥ 9 years of age, ***Season***: corresponding to the wet winter months (June–August), spring (September – November), the dry summer months (December – February) and autumn (March – May). I further analysed the GAS results according to the residence of the participants (as opposed to by clinic) given that patients do not necessarily attend the clinic nearest to their homes.

The annual rate of incident cases of GAS pharyngitis was calculated on a gender- and community-specific basis using an estimate of the number of children aged five to fifteen years in the catchment areas based on census data for the Vanguard population (Statistics South Africa, 2003) – see Section 2.1. Incidence data are thus presented for the 5 – 15 age groups residing within the Bonteheuwel and Langa communities. We revised the

yearly population total for each year of the study taking into account an annual population growth of 1% per annum (World Bank, 2012). Significance was accepted at the two-sided level of 0.05. Statistical analyses were performed using STATA version 11.0 (SPSS Inc., Chicago, IL, USA).

5.2.6 Ethical Approval

The University of Cape Town's Human Research Ethics Committee approved the study. We also received permission from the various local departments of health as well as from the management of each facility.

5.3 RESULTS

5.3.1 Enrolment and Demographics

From May 2008 to April 2012, 902 children were evaluated for eligibility to participate in the study. Following exclusion due to recent antibiotic use (n=35), age ineligibility (n=1), refusal on the part of the parent or child (n=16) or incomplete data (n=10), 840 participants were enrolled over the 4-year period (Table 5.1). Female children were in the majority with 468 cases (56%). The mean age amongst all participants was 8.17 years (range 3 – 15 years). Children under the age of nine years presenting with sore throat to the community clinics were more likely to be female (O.R 1.41, 95% CI 1.07-1.86).

The majority of participants (72.1%) was enrolled from Bonteheuwel, 18% from Langa and 9.8% from outside the Vanguard area. Of the three clinics, the Vanguard Community Health Centre with 639 patients had the highest number enrolled while the

TABLE 5.1 **Demographic Data for Enrolled Participants According to Clinic**

Demographic		Total	Clinic		
			Vanguard	Langa	Netreg
Participants, n (%)		840 (100)	639 (76)	92 (11)	109 (13)
Sex	Female	468 (56)	351 (55)	48 (52)	69 (63)
	Male	372 (44)	288 (45)	44 (48)	40 (37)
Community	Bonteheuwel	606 (72)	567 (89)	5 (5)	34 (31)
	Langa	152 (18)	65 (10)	87 (95)	0 (0)
	Other	82 (10)	7 (1)	0 (0)	75 (69)
Age, y§	Mean (+/- 2sd)	8.2 (2.5)	8.1 (2.5)	8.6 (2.6)	8.0 (2.6)
	3 - 8	466 (55)	365 (57)	43 (47)	58 (53)
	9 - 15	374 (45)	274 (43)	49 (53)	51 (47)
Study Period	1. May 08 – Apr 09	286 (34)	246 (39)	27 (25)	13 (14)
	2. May 09 – Apr 10	177 (21)	127 (20)	25 (23)	25 (27)
	3. May 10 – Apr 11	197 (24)	123 (19)	35 (32)	39 (43)
	4. May 11 – Apr 12	180 (21)	143 (22)	22 (20)	15 (16)
Season§§	Winter (June – Aug)	211 (25)	190 (30)	9 (10)	12 (11)
	Spring (Sept – Nov)	240 (29)	170 (26)	36 (39)	34 (31)
	Summer (Dec – Feb)	147 (17)	94 (15)	23 (25)	30 (28)
	Autumn (Mar – May)	242 (29)	185 (29)	24 (26)	33 (30)

n, number; y, years; sd, standard deviation;
§P value was significant for χ^2 test assessing difference in distribution of age category w.r.t gender (p = 0,0138)
§§ Seasons are designated according to the local climate with summer and winter being the driest and wettest seasons respectively

Netreg clinic had the highest female to male ratio (female to male ratio 1.7:1). Over the four years of the study, overall enrolment was highest during the first year of the study (May 2008 – April 2009). Netreg clinic's recruitment, though, was the most in the third year of study. Peak season of enrolment for the Langa and Netreg clinics was spring, while for Vanguard, winter had the highest numbers recruited.

5.3.2 Period prevalence of GAS Pharyngitis

Table 5.2 shows the demographic and GAS status of the laboratory cultures investigated over the study period. Overall, GAS was positive in 181 of the 840 patients (21.6%) presenting with pharyngitis as a symptom to the community clinics with no significant differences found with respect to age categories (< 9 or ≥ 9) or gender. Isolation rates for GAS were similar across the three communities ranging from 21% to 23%. The highest proportion of GAS isolates occurred during the third study period (2010 – 2011) with 55 participants (28%) being positive from the 197 enrolled. Autumn had the highest proportion (27%) of GAS isolates while in Winter, only 15% of throat swabs were positive for the organism. There was no plausible explanation for the drop in the numbers of children enrolling year by year; the study is ongoing and trends will continue to be monitored in the coming years.

5.3.3 Recurrent Episodes of Culture–Positive Pharyngitis

Fifty-four children had repeated episodes of pharyngitis with 46, 5 and 3 children having 2, 3 and 4 episodes of pharyngitis, respectively. Of these, 6 children had two episodes of GAS positive pharyngitis.

TABLE 5.2 **Comparison of GAS-Positive Throat Cultures According to Community**

Demographic		Total	Bonteheuwel	Langa	Other
GAS+ / Participants, n (%)		181/840 (22)	128/606 (21)	34/152 (22)	19/82 (23)
Gender	Female	103/468 (22)	69/335 (21)	24/81 (30) §	10/51 (20)
	Male	78/372 (21)	59/270 (22)	10/71 (14)	9/31 (29)
Age, y	3 - 8	91/466 (20)	67/350 (19)	14/74 (19)	10/42 (24)
	9 - 15	90/374(24)	21/256 (24)	20/78 (26)	9/40 (23)
Study Period	1. May08 – Apr09	47/286 (16)	34/227 (15)	12/55 (22)	1/4 (25)
	2. May09 – Apr10	39/177 (22)	30/114 (26)	4/39 (10)	5/24 (21)
	3. May10 – Apr11	55/197 (28)	33/123 (27)	13/42 (31)	9/32 (28)
	4. May11 – Apr12	40/180 (22)	31/142 (22)	5/16 (31)	4/22 (18)
Season	Winter	32/211 (15)	23/169 (14)	5/28 (18)	4/14 (29)
	Spring	51/240 (21)	34/162 (21)	12/60 (20)	5/18 (28)
	Summer	33/147 (22)	24/104 (23)	6/24 (25)	3/19 (16)
	Autumn	65/242 (27)	47/171 (27)	11/40 (28)	7/31 (23)

n, number; y, years; § P value was significant for χ^2 test assessing difference in distribution of gender in Langa participants w.r.t. GAS positivity (p=0,022)

5.3.4 Period prevalence of Streptococcal Groups other than GAS

Non-GAS streptococcal isolates were recovered from 62 children: these included group C (32), group D (1), group F (5) and group G (24).

5.3.5 Demographic Associations Culture-Positive Sore Throat

There were no significant associations between demographic variables and GAS positivity with one exception: females from Langa were more likely to have a GAS-positive throat culture than male children (O.R 2.568, 95% CI 1.11-5.94), Figure 5.1; this was observed in three of the four years of the study period. In the second year of the study, there was a significantly higher rate of GAS isolation from participants from Bonteheuwel compared

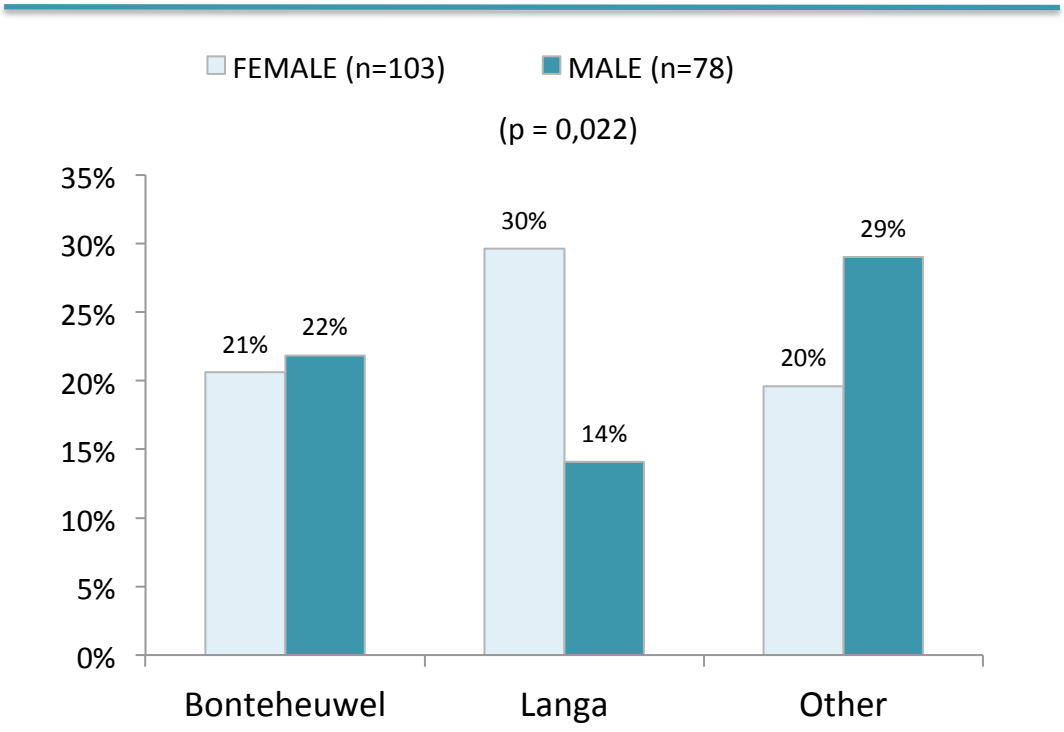

Figure 5.1. Gender-specific GAS isolation rates by community within Vanguard Demonstration Site

with Langa, p=0.037. The analysis of GAS isolation by season across the four study years is presented in Table 5.3. Overall, there was no significant difference in the rates of isolation on a year-by-year basis, p values = 0.132, 0.190, 0.203 respectively. When considering seasons, autumn had the highest proportion of GAS isolated ranging from 20.7 - 37.3% over each of the first three years of the study (Figure 5.2.). During year 4, the highest percentage of GAS was isolated in summer 10/33 (30.3%).

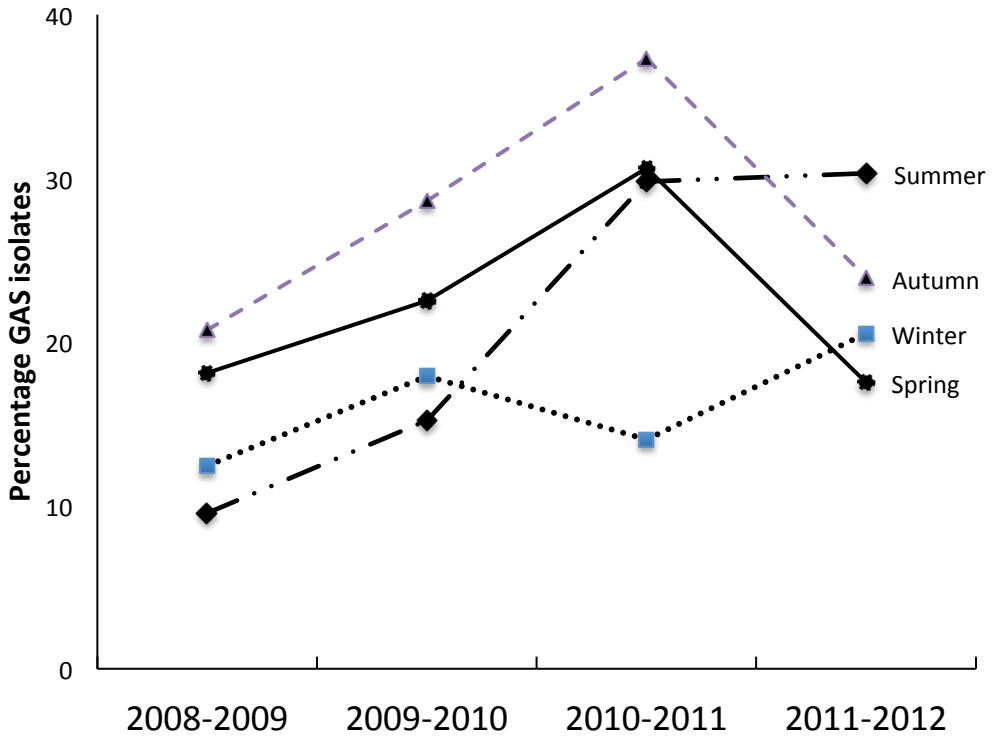

Figure 5.2. Seasonal variation in the proportion of culture-positive GAS isolated in the Vanguard Demonstration Site within each year

TABLE 5.3 **Comparison of GAS-Positive Throat Cultures According to Study Year**

Year	No of Children (%)	No (%) of GAS-positive cultures	*P-value*
2008 - 2012	840	181 (22)	
Year 1: 2008-2009	286	47 (16.4)	- - -
Winter	89 (31)	11 (12.4)	
Spring	94 (33)	17 (18.1)	
Summer	21 (7)	2 (9.5)	
Autumn	82 (29)	17 (20.7)	
Year 2: 2009-2010	177	39 (22)	0.132
Winter	28 (16)	5 (17.9)	
Spring	40 (23)	9 (22.5)	
Summer	46 (26)	7 (15.2)	
Autumn	63 (36)	18 (28.6)	
Year 3: 2010-2011	197	55 (28.0)	0.190
Winter	50 (25)	7 (14.0)	
Spring	49 (25)	15 (30,6)	
Summer	47 (24)	14 (29.8)	
Autumn	51 (26)	19 (37.3)	
Year 4: 2011-2012	180	40 (22.2)	0.203
Winter	44 (24)	9 (20.5)	
Spring	57 (32)	10 (17.5)	
Summer	33 (18)	10 (30.3)	
Autumn	46 (26)	11 (23.9)	

No, number; P value for χ^2 test assessing difference in distribution year on year

5.3.6 Incidence Rates for Sore Throat and GAS Pharyngitis

Based on the number of cases and age-specific population data, the incidence of new cases of pharyngitis for the population of the Vanguard community aged 5-15 years was estimated to be 0.837 cases/100 person-years (Table 5.4). The incidence of sore throat varied according to age, being higher among 5-9 years of age than among those aged 10-15 years (IRR 2.341, 95% CI 2.00-2.74).

The incidence of GAS pharyngitis for the population of the Vanguard Demonstration site aged 5-15 years was 0.18 cases/100 py. GAS sore throat incidence ranged from 0.14 – 0.21 per 100 py over the respective years of the study. A significantly higher incidence of GAS pharyngitis was observed in the younger age group (I.R.R 2.265, 95% CI 1.61-3.21), a trend seen throughout each year of the study period (Figure 5.3).

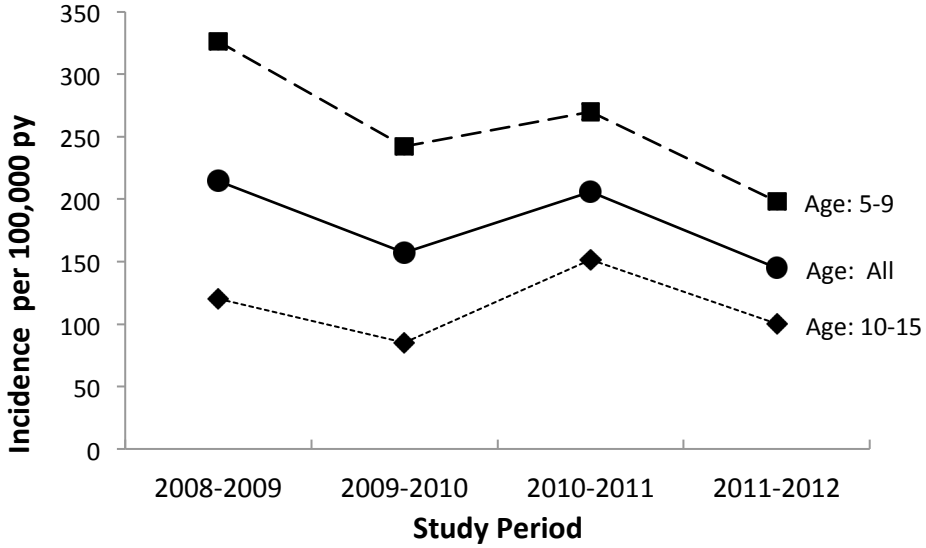

Figure 5.3. Estimated incidence of GAS culture-positive pharyngitis in Bonteheuwel and Langa children

TABLE 5.4 **Estimated Incidence of Sore Throat and Primary Cases of GAS Culture-Positive Pharyngitis**

Parameter	Sore Throat				GAS Pharyngitis			
	Cases, *n*	Time at risk, py	Incidence per 100 py	95% CI	Cases, *n*	Time at risk, py	Incidence per 100 py	95% CI
Overall	728	87040	0.836	0.77 – 0.89	157	87040	0.180	0.15 – 0.21
Age, y								
5 – 9	483	39789	1.214	1.11 – 1.33	103	39789	0.259	0.21 – 0.31
10 – 15	245	47251	0.519	0.46 – 0.59	54	47251	0.114	0.086 – 0.149
Study Period								
Year 1	282	21436	1.316	1.17 – 1.48	46	21436	0.215	0.16 – 0.29
Year 2	153	21651	0.707	0.59 – 0.83	34	21651	0.157	0.11 – 0.22
Year 3	161	21867	0.736	0.63 – 0.86	45	21867	0.206	0.15 – 0.28
Year 4	132	22086	0.598	0.50 – 0.71	32	22086	0.145	0.099 – 0.201

n, number; py, person-years; CI, confidence interval; y, years;

5.4 DISCUSSION

To the best of my knowledge, this is the first prospective incidence study of sore throat and GAS pharyngitis in Africa. Participants for this study were enrolled over four years from the three state-funded facilities in the Cape Town suburbs of Bonteheuwel and Langa. The incident rate per 100 py for sore throat presentation was 0.836 cases and 0.180 for GAS pharyngitis.

We calculated incidence rates using population-based regional census data. Our study presents incidence rates noticeably lower than those previously reported. The incidence rates of GAS pharyngitis episodes among 5 to 15 year-olds ranged from 0.157 to 0.215 per 100 py over the individual years of the study. Incidence rates of GAS pharyngitis from North India were estimated to be 950 / 10^3 py (Nandi et al., 2001), Czechoslovakia, 39 / 1,000 py (Duben et al., 1979) and Melbourne, 130 / 1,000 py (Danchin et al., 2007) and 140.7 / 1,000 py cases (Steer et al., 2009). The lower incidence in our study may indicate that, either episodes of group A streptococcal pharyngitis are exceedingly uncommon in the Vanguard Community or that presentation with this condition is exceedingly uncommon. Either way, this finding has important implications for policy, especially as regards whether the focus of prevention efforts for rheumatic fever should be on primary prevention. It is likely that our incidence rates are an underestimate of the true effect, especially given that our denominator potentially included children who do not use the state facilities; our study has not accounted for the small proportion of children who attend private general practitioners for care.

The period prevalence of GAS pharyngitis in patients with sore throat was 22% across the three participating clinics. GAS isolation rates were similar for participants irrespective of

place of residence; despite significant differences in SES indicators between the two suburbs of Langa and Bonteheuwel. Other studies have also reported a negligible effect of SES on GAS results (Nandi et al., 2001, Danchin et al., 2007). In an earlier study in Pretoria, South Africa, 33,2% of cases of pharyngitis in 5-25 year old patients were GAS-positive and the notable difference noted between the GAS isolation rates between black and white participants were postulated to be attributed to the differences in the socio-economic conditions across race groups within South Africa at the time (van Zyl et al., 1981). We could not explain the higher rate of GAS recovery amongst female children as compared with male children residing in Langa, a finding not consistent with results from other studies. Concerning isolates other than GAS, we found a low period prevalence of non-GAS isolates among our cohort. Unfortunately, the numbers are too low to investigate any associations between clinical parameters examined and the type of organism isolated.

Children aged 5 – 9 years had incidence and period prevalence rates of more than twice those of children aged 10 years or older. This is in keeping with data reported elsewhere for both sore throat (Nandi et al., 2001, Duben et al., 1979) and GAS-positive pharyngitis (Steer et al., 2009, Danchin et al., 2007).

The rates of GAS isolation was highest in the autumn months for three of the four years of the study period, with summer having the peak prevalence in the remaining year. While this finding differs from reports of high prevalence being frequently associated with the wet winter months, when children are more likely to spend time indoors in crowded surroundings, thus increasing the probability of transmission of infection (Sarkar et al., 1988), others have reported GAS isolates being recovered across all the seasons.

Our study has a number of strengths: The participants were enrolled at the time of presentation of sore throat at the clinic, thus reducing potential bias which may come from patients knowing that they are being followed for repeated episodes of pharyngitis. Also, the passive approach to participant recruitment made it less likely that GAS carriers would have been inadvertently enrolled.

However, limitations in this study as far as enrolment is concerned include a likelihood of missing children who are privately insured for medical treatment, or missing children who sought health care after hours, given that our study was limited to normal office hours. However, this was likely to be minimal. Another limitation in this study was the changeover of nursing staff, with four nurses participating in the study over the study period. Again, though we expect this effect to be minimal given that the latest nurse has been on the project for > 2 years.

These data confirm that the complaint of sore throat is common, particularly amongst younger children. While relatively lower than prevalence and incidence rates of GAS reported elsewhere, the numbers nevertheless call attention to the need for vigilance on the correct management of sore throat.

6 CLINICAL PREDICTION OF GAS PHARYNGITIS: STUDY FOUR

STREPTOCOCCAL PHARYNGITIS IN CHILDREN PRESENTING WITH SORE THROAT: EVALUATION OF EXISTING CLINICAL PREDICTION RULES AND DEVELOPMENT OF A CAPE TOWN STREP THROAT SCORE

6.1 INTRODUCTION

Antibiotic therapy for acute streptococcal pharyngitis has been proven to be effective in decreasing the complication of ARF: in a meta-analysis of 10 studies involving in excess of 7500 participants, Robertson et al determined a 68% risk reduction in the incidence of ARF in those receiving antibiotics compared with controls (Robertson et al., 2005a). As discussed previously (Section 1.6), in South Africa, the management of pharyngitis (including tonsillitis) is based on a diagnosis of streptococcal pharyngitis made on suggestive clinical features, without the benefit of microbiological culture confirmation or rapid antigen testing (Department of Health, 1999). Thus, a risk assessment using an accurate, objective model of clinical prediction derived from health facility-based data is of vital importance to the primary-care physicians and nursing practitioners in making management decisions.

As reviewed in Section 1.4, it is preferable that a clinical prediction rule (CPR) is tested or validated before being adopted into practice (Wasson et al., 1985, McGinn et al., 2003). An evaluation of CPRs for the management of streptococcal pharyngitis developed largely in industrialised nations showed poor performance in populations different from those in

which they were developed especially in low-income regions of the world (Fischer Walker et al., 2006, Rimoin et al., 2005). A recent systematic review of fifteen studies confirmed the inability of a selection of five CPRs to confidently rule in a diagnosis of streptococcal pharyngitis (Shaikh et al., 2011). No decision rules have been developed in sub-Saharan Africa and thus, it is imperative to evaluate existing CPRs for GAS pharyngitis within the local context.

The objective of this study was twofold: first, we wished to test the generalizability of three existing CPRs namely, the World Health Organisation (WHO) Acute Respiratory Infection (ARI) case management strategy (WHO, 1995), the 3-variable Steinhoff rule (Steinhoff et al., 2005) developed in an outpatient facility in Cairo (and thus bearing resemblance to African conditions and the McIsaac Rule (McIsaac et al., 1998) in patients aged 3-15 years presenting with pharyngitis in the primary care setting; second, to develop a locally applicable CPR to assist primary care physicians and nursing practitioners in identifying children, aged 3-15 years, who are at risk for streptococcal pharyngitis.

6.2 PATIENTS AND METHODS

The methods are described in detail in Section 2.3 'Rationale and Design of the studies".

6.2.1 Participant Recruitment

Participants for this prospective study were recruited over four years from May 2008 from three clinics serving the Vanguard community of Cape Town following informed consent according to a standard protocol. Prior to obtaining the throat swab, the research nurse recorded, in addition to the demographic characteristics of participants, recent antibiotic

usage, the date of presentation at the clinic and symptoms evaluated at the time of clinical examination. Together with age and gender, the following 10 symptoms and signs were recorded as absent or present: cough, rhinorrhoea, hoarseness, temperature above 38°C, tonsillar erythema and swelling, presence of exudate on the pharynx or tonsils, oropharyngeal candidiasis, tenderness of an anterior cervical lymph node on palpation, presence of an anterior cervical lymph node greater than 1.5cm in diameter and/or presence of a rash.

Microbiological procedures have been described previously in Section 2.3.6 (Specimen Collection, Transport and Handling).

6.2.2 Data Analyses

Statistical analysis was performed using Stata version 11.0 (SPSS Inc., Chicago, IL, USA) Proportions or means were calculated to describe the baseline characteristics of the study population. Laboratory-confirmed GAS culture positivity was regarded as being indicative of streptococcal pharyngitis in assessing the respective rules. Based on the prospective data collected, we generated scores as defined in the respective rules to estimate the probability of a GAS positive culture (Box 1.2). The WHO rule predicts GAS positivity on the presence of exudate on the pharynx and a large tender anterior cervical node on palpation while for the Steinhoff rule, a score is calculated from 1-3, one point being given for each of enlarged cervical node, absence of rhinitis or absence of rash. We used the recommended cut-off of 2, which corresponds to a sensitivity of 92% and specificity 38% for the detection of GAS positivity. The McIsaac rule which renders a score from zero to five, in addition to the clinical variables of temperature > 38^{o}C, tender anterior cervical adenopathy, tonsillar swelling or exudate and absence of cough, also incorporates the age of the patient into the

decision-making with under 15 years of age, 15-44 years and ≥ 45 years contributing scores of 1, 0 and -1 respectively. For each rule, we calculated sensitivity ("true positive rate") and specificity ("true negative rate"), the positive predictive value (PPV) and negative predictive value (NPV), although these have the limitation of fluctuating as the prevalence of disease and likelihood ratios in a population change. In addition, we determined the numbers of missed streptococcal cases (MDx) and unnecessarily treated non-cases (WDx) in order to calculate the ratio of MDx / WDx (Steinhoff et al., 2005). The lower the MDx/WDx, the better the performance of the test.

For developing the CPR, we required 10 events per candidate predictor (index test) (Peduzzi et al., 1996). For illustration purposes, we calculated univariable odds ratios with 95% confidence intervals to examine the relationship between each individual predictor with the primary outcome measure, i.e. a laboratory-confirmed GAS culture from the throat swab specimen. For continuous predictors, the form of association with the outcome was checked using restricted cubic splines, to find the proper transformation if needed (Harrell, 2001). No selection of predictors was made on these given the potential of creating unstable prediction models (Sun et al., 1996). We then entered all the candidate predictors (index tests) into a multivariable logistic regression model, with GAS as the primary outcome measure, to identify which of the candidate predictors independently contributed to the inclusion or exclusion of the diagnosis GAS positivity. Age was modelled as a dichotomous variable below or above the median value. A backward elimination modelling technique was used, with a significance limit for removal from the model of $P>0.10$ on the basis of the likelihood ratio test. We chose not to use a p-value of < 0.05 as a cut-off given the potential for poor model performance when tested on new

patients (Janssen et al., 2010). The new CPR rule was constructed incorporating previously described methods (Zuithoff et al., 2009, McIsaac et al., 1998) where the logistic formula was transformed into a ready to use score chart. Beta coefficients were divided by a constant; predictors with a negative association were inverted and evaluated as positive entities; the total score was then compared to the observed proportion of GAS positivity in our cohort. Receiver operating curves (ROC), together with a plot of sensitivity versus specificity were generated to evaluate the predictive power of the model.

6.2.3 External Validity

To determine generalizability of the new CPR to new patients, the rule was applied to both the derivation set and a new data set comprising children from a subsequent period in the same group of clinics (validation set) as per recommendations in the literature (Bleeker et al., 2003). For comparison purposes, we also applied the same CPRs originally evaluated on the derivation set as discussed above. Data collection, definition of clinical variables, and laboratory procedures to determine GAS status among the validation cohort were identical to the original protocol and performed by the same study nurse.

6.3 RESULTS

Derivation set

Over the first three years of our study, 720 children with pharyngitis were examined by the study nurse across the three study clinic sites. Of these, informed consent was obtained for 666 participants who met the inclusion criteria and were recruited into the study; this cohort was used to evaluate the existing CPRs and derive the new CPR (derivation set).

Reasons for exclusion were antibiotic treatment in the preceding 30 days, incorrect age and refusal by the child to allow a throat swab to be taken. The age of the patients ranged from 3-15 years with a mean age of 8.3 years (sd, 2.37 years). The proportion of males was 43.4%. Males and females differed as regards the mean age of presentation (males 7.96 years vs females 8.61 years; $p<0.01$). A laboratory-confirmed diagnosis of GAS was reported in 142 patients (21.3%), which exceeded the 115 cases as per the sample size calculated to develop a CPR (Section 2.3.8.2). Tonsillar erythema and swelling were present in more than 80% of participants, while oral candidiasis was seen rather infrequently (<5%).

Validation set

Participants recruited from May 2011 until April 2012 constituted the validation set, comprising 172 participants recruited in the same manner, which was used to evaluate the performance of the newly derived CPR. The age of participants ranged from 3-15 years with a mean age of 7.45 years (sd, 2.93 years). Of these, a positive GAS diagnosis was reported in 38 (22.1%) participants. Tonsillar swelling and tonsillar erythema were the predominant clinical symptoms, while presence of an exudate on the pharynx and oropharyngeal candidiasis were absent. The baseline characteristics of the participants from the derivation and validation sets are presented in Table 6.1. The baseline characteristics amongst the validation cohort were not significantly different from the derivation set.

Table 6.1: **Comparison of the characteristics of patients presenting with pharyngitis in the derivation and validation sets**

DataSet	Derivation	Validation	*p-value*
Sample Size	**666**	**172**	
Baseline Characteristics	**n (%)**	**n (%)**	
Age > 8 years	305 (45.8)	66 (38.4)	*0.081*
Male Gender	289 (43.4)	86 (50.0)	*0.120*
Laboratory-confirmed Group A *Streptococcus* +	142 (21.3)	38 (22.1)	*0.826*
Clinical Symptoms			
Cough	455 (68.3)	120 (69.8)	*0.715*
Rhinorrhea	394 (59.2)	94 (54.7)	*0.285*
Hoarseness	162 (24.3)	2 (1.2)	*<0.001*
Temperature > 38 ^{o}C	134 (20.1)	105 (61.1)	*<0.001*
Tonsillar erythema	547 (82.1)	171 (99.4)	*<0.001*
Tonsillar swelling	545 (81.8)	172 (100)	*<0.001*
Presence of exudate on the pharynx	76 (11.4)	0 (0.0)	-
Presence of exudate on the tonsils	327 (49.1)	102 (59.3)	*0.017*
Oropharyngeal candidiasis	28 (4.2)	0 (0.0)	*0.006*
Tenderness of an anterior cervical node	348 (52.3)	123 (71.5)	*<0.001*
Presence of an anterior cervical lymph node greater than 1.5 cm in diameter	153 (22.9	125 (72.7)	*<0.001*
Presence of a rash.	114 (17.1)	4 (2.3)	*<0.001*

6.3.1 Evaluation of Existing CPRs

A summary of the diagnostic performance of three decision rules in the 666 children comprising the derivation set is provided in (Table 6.2). Compared with laboratory-confirmed culture results, the following values were obtained for sensitivity and specificity respectively: 8.5% and 93.3% for the WHO ARI guideline, 54.9% and 54.2% for the Steinhoff rule and 38.0% and 77.5% for the McIsaac rule. The Steinhoff rule showed the best performance with a positive likelihood ratio of 1.20.

6.3.2 Development of a Locally Applicable CPR

Table 6.3 shows the results of the association between throat culture results and clinical findings of all 14 variables in the derivation data set. Tonsillar swelling ($P < 0.001$), was the strongest univariate predictor of a positive GAS culture. A further four variables were significant predictors for GAS positivity: a temperature > 38 ^{o}C ($P=0.007$), presence of exudate on the tonsils ($P=0.001$), tenderness of an anterior cervical node on palpation ($P=0.026$) and the presence of an anterior cervical lymph node > 1.5 cm in diameter ($P=0.036$). Strong predictors inversely associated with GAS positivity were cough ($P < 0.001$), rhinorrhoea ($P=0.002$), and presence of a rash ($P = 0.039$).

Table 6.2: **Performance of 3 clinical decision rules for streptococcal pharyngitis in children aged 3-15 years comprising the derivation set (n=666) from the Vanguard Demonstration Site**

Decision Rule	Sens	Spec	PPV	NPV	LR+	MDx (%)	WDx (%)	MDx/WDx
WHO Rule	8.5	93.3	25.5	79.0	1.27	130 (91)	35 (7)	3.71
Steinhoff rule (at cut-off 2)	54.9	54.2	24.5	81.6	1.20	64 (45)	240 (46)	0.27
McIsaac Rule (at score ≥ 4)	38.0	77.5	31.4	82.2.0	1.69	88 (62)	118 (23)	0.75

Sens, sensitivity; Spec, specificity; PPV, positive predictor value; NPV, negative predictive value all expressed as percentages; MDx, number of missed streptococcal cases; WDx, number of unnecessarily treated non-cases.

Table 6.3: **Results of Univariable Analysis of Potential Predictive Variables for GAS in children aged 3-15 years with pharyngitis: Derivation Set**

Variable		GAS+ Patients with variable, n *(%)*		Unadjusted OR for GAS+ (95% CI)	*P* value
Age > 8 years		72	*(50.7)*	1.28 (0.87 – 1.86)	0.186
Male Gender		55	*(38.7)*	1.27 (0.87 – 1.86)	0.291
Cough		78	*(54.9)*	0.47 (0.32 – 0.70)	< 0.001
Rhinorrhea		68	*(47.9)*	0.56 (0.38 – 0.81)	0.002
Hoarseness		29	*(20.4)*	0.75 (0.48 – 1.19)	0.22
Pyrexia > 38 °C		40	*(28.2)*	1.79 (1.16 – 2.75)	0.007
Tonsillitis	erythema	126	*(88.7)*	1.92 (1.09 – 3.38)	0.022
	swelling	132	*(92.9)*	3.55 (1.80 – 6.98)	< 0.001
	exudate	88	*(61.9)*	1.94 (1.33 – 2.84)	0.001
Exudate on the pharynx		16	*(11.3)*	0.98 (0.55 – 1.76)	0.952
Oropharyngeal candidiasis		6	*(4.2)*	1.00 (0.40 – 2.53)	0.93
Cervical glands	Enlarged	42	*(29.6)*	1.56 (1.03 – 2.37)	0.036
	Tenderness	86	*(60.6)*	1.54 (1.05 – 2.24)	0.026
Presence of a rash.		16	*(11.3)*	0.55 (0.31– 0.97)	0.039

Table 6.4 details the multivariate analysis describing the variables and the corresponding odds ratio, as well as the beta-coefficients and p values. Our multivariable logistic regression model consisted of the following predictors: cough (OR, 0.59, 95% CI 0.40 – 0.90), rhinorrhoea (OR, 0.66, 95% CI 0.45;0.99), tonsillar swelling (OR, 2.98, 95% CI 1.49;5.93), tenderness of an anterior cervical node on palpation (OR, 1.55, 95% CI 1.05;2.32) and rash (OR, 0.54, 95% CI 0.30;0.99). The area under the curve of the model was 0.67.

Table 6.4: **Multivariable Analysis of Potential Predictive Variables for GAS in children aged 5-15 years with pharyngitis**

Variable	Adjusted Odds Ratio (95% CI)	Beta	(*P* value)
Tonsillar swelling	2.98 (1.49 – 5.93)	1.091	0.002
Tender anterior cervical node	1.55 (1.05 – 2.32)	0.443	0.029
Rhinorrhea	0.66 (0.45 – 0.99)	- 0.406	0.046
Cough	0.59 (0.40 – 0.90)	- 0.514	0.013
Rash	0.54 (0.30 – 0.99)	- 0.611	0.042

Table 6.5 illustrates the scoring system developed to calculate the risk of GAS positivity in a child presenting with sore throat. Clinical predictors were weighted equally with a simple one-point being allocated for each predictor value, and summed into a score out of 5. The observed proportions of patients with GAS positivity and corresponding score categories in each of our derivation and validation cohorts are shown in column 'observed proportion'

Table 6.5: **Scorecard based on multivariable analysis model to calculate the probability of GAS positivity in children aged 3 - 15 years with pharyngitis**

Clinical sign	Score
Presence of tonsillar swelling	1
Presence of tender anterior cervical node	1
Absence of cough	1
Absence of rhinorrhea	1
Absence of rash	1
Maximum Score	**5**

in Table 6.6. Using the validation set, 71.05% of all patients obtained a score ≥ 3 using the newly-derived scorecard; thus, we evaluated the discriminatory ability of the new rule at a cut-off of ≥ 3. We also contrasted these findings with the existing rules evaluated in the derivation set of our study (Table 6.7).

Table 6.6: **Observed percentage of GAS-positive patients within a specific range of scores**

Total Score	Derivation set (n=666)		Validation set (n=172)	
	GAS Count (%)	% of all patients with score	GAS Count (%)	% of all patients with score
5	21 (14.8)	14.8	2 (5.26)	5.26
4	48 (33.8)	48.6	9 (23.68)	28.95
3	43 (30.3)	78.9	16 42.11)	71.05
2	26 (18.3)	97.2	11 (28.95)	100.0
1	4 (2.8)	100.0	-	-

Table 6.7: **Performance of the Cape Town Clinical Prediction Rule for streptococcal pharyngitis using a cut-off score of ≥ 3 in children aged 3 - 15 years in the Vanguard population validation set. Existing rules were evaluated as a comparison.**

Decision Rule	Sens (95% CI)	Spec (95% CI)	PPV	NPV	LR+	MDx (%)	WDx (%)	MDx/WDx
VDS rule (at cut-off 3)	71.1 (54.1; 84.6)	12.7 (7.6; 19.5)	18.8	60.7	0.81	11 (29)	117 (87)	0.09
Steinhoff rule (at cut-off 2)	65.8 (48.6; 80.4)	13.4 (8.2; 20.3)	17.7	58.1	0.76	13 (34)	116 (87)	0.11
McIsaac Rule (at score ≥ 4)	28.9 (15.4; 45.9)	32.8 (24.9;41.5)	10.9	62.0	0.43	27 (71)	90 (67)	0.30

Sens, sensitivity; Spec, specificity; CI, confidence interval; PPV, positive predictor value; NPV, negative predictive value all expressed as percentages; MDx (%), number of missed streptococcal cases; WDx, number of unnecessarily treated non-cases.

6.4 DISCUSSION

The potential of reducing ARF in areas of high incidence requires the appropriate diagnosis and treatment of streptococcal pharyngitis without necessarily relying on laboratory confirmation of GAS positivity (Karthikeyan and Mayosi, 2009). A number of CPRs for predicting streptococcal pharyngitis have been published and tested over the years with varying results in different populations. This study, the first in sub-Saharan Africa, sought to evaluate and compare the effectiveness of three existing clinical rules for managing children with GAS-positive pharyngitis, and if found to have low sensitivity, to develop a locally applicable CPR. The existing CPRs have a low sensitivity for detecting GAS pharyngitis in Cape Town. We have developed a new CPR with high sensitivity that may be more applicable in sub-Saharan Africa

We chose three CPRs purported to identify patients with GAS positivity: the ARI guideline advocated by the WHO (WHO, 1995), the McIsaac rule which incorporates variables which were included in our study (McIsaac et al., 1998), and the Steinhoff rule, which was developed in a low-income setting (Steinhoff et al., 2005), and the only rule developed on the African continent and bearing resemblance to our setting. In evaluating effectiveness of these, we used sensitivity and specificity as the main measure of performance. We provide the positive predictive and negative predictive values as well as the numbers and percentages of failed diagnostic cases and incorrectly treated non-cases. In addition, we also calculated the ratio of missed diagnosis cases (MDx) to incorrectly treated non-cases (WDx) to allow comparison amongst the rules and with published data. Of the three rules tested, the McIsaac Rule using a cut-off of ≥ 4, had the highest positive predictive value, but missed 62% of the culture-positive children who should have been

treated. By the same criteria, 22.5% of non-cases would have been prescribed antibiotics thus, increasing inappropriate use of antibiotics. The Steinhoff rule had the best positive likelihood ration (LR+) with a sensitivity of 54.9% in our cohort; however, in excess of 45% of patients would have been unnecessarily treated. Our findings regarding the dismal performance of the WHO guideline were similar to previous reports from low-resource settings (Rimoin et al., 2005).

We performed logistic regression to determine the potential contribution of various clinical parameters in aiding a diagnosis of GAS pharyngitis amongst our participants. Not all of the clinical parameters in our study, which were compiled from a variety of existing clinical prediction rules, were included in the multivariate model. Our analysis identified five clinical considerations to be taken into account when determining the likelihood of GAS positivity in the absence of laboratory confirmation; these included the presence of tonsillar swelling, a tender anterior cervical lymph node, absence of cough, absence of rhinorrhoea, and absence of rash. These variables were largely similar to those incorporated within the three rules evaluated. The presence of exudate on the pharynx and a temperature > 38^{o}C, criteria in the WHO guideline and the McIsaac rule respectively were however, not included in the final model.

The proposed score chart was externally validated with participants from a different time period than the derivation set. To allow for comparison, we also included the existing rules tested earlier on the derivation set. The WHO guideline, unfortunately, could not be included since the participants in the validation set showed no symptoms of exudate on the pharynx, a requirement for the WHO rule. Our newly-derived Cape Town Clinical

Prediction Rule for GAS pharyngitis (at a cut-off of 3) had a sensitivity of 71% and a LR+ of 0.81 which, in comparison to the existing rules, proved to be the best performer.

There is debate as to the acceptable level of false-negative rates of CPRs for GAS pharyngitis. Taking into account the rarity of reports of penicillin anaphylaxis and absence of microbial resistance, it is desirable to employ CPRs demonstrating a low MDx/WDx ratio which indicates favouring less missed diagnosis over incorrectly treated non-cases. We included the MDx/WDx ratio to allow us to compare the different CPRs against each other. In both our derivation and validation cohorts, of the three existing rules evaluated, the Steinhoff rule had the lowest MDx/WDx ratio confirming its suitability for our context despite the potential selection bias in their cohort where pharyngeal erythema needed to be present. In line with our expectation though, the Cape Town Clinical Prediction Rule (at a cut-off of 3) had the lowest MDx/WDx ratio when considering the Steinhoff and McIsaac rules.

This study had a variety of strengths: sufficient events were included in developing the rule; the datasets were complete and no imputation for missing data was needed; model assessment was conducted on a separate dataset, the validation set comprising participants enrolled from the same area and with the same protocol as for the derivation set, thus ensuring continuity in the examination procedure.

Furthermore, given that participants were enrolled from amongst symptomatic patients seeking care at the community clinic, the probability of having enrolled asymptomatic patients is reduced. Thus, there is no need to establish the carrier rate in asymptomatic individuals from the sampled population proposed by others (McGinn et al., 2000).

In conclusion, we developed a prediction model for GAS pharyngitis in children aged 3-15 years in low-resource settings. The model development fulfils many of the recommendations for building a multivariate prognostic model using regression techniques with empirical data. While prospective studies utilising health practitioners in primary health care clinics are required to evaluate applicability in other low-resource settings, our rule nevertheless proved vastly superior to the recommended WHO ARI guideline. Furthermore, our rule also performed marginally better than the Steinhoff rule, the only other rule developed on the African continent in a similar setting to ours. Using such a model may help to reduce the incidence of ARF and RHD in the long term.

7 PREVALENCE OF RHD: STUDY FIVE

PREVALENCE OF RHEUMATIC HEART DISEASE IN THE VANGUARD COMMUNITY DEMONSTRATION SITE, SOUTH AFRICA

7.1 INTRODUCTION

The literature review for this chapter is covered in details in Section 1.5.3. Mortality rates for RHD range from 1.2 to 23.8 per 100,000, being highest among the indigenous communities of Australia and lowest in industrialised countries (Jackson et al., 2011). In addition, up to 80 million people are affected with RHD (Paar et al., 2010), and 1 million are in need of open-heart surgery and valve replacement, which is often not feasible in many developing countries with a heavy burden of RHD (Mocumbi, 2012).

7.1.1 Echo-Based School Screening Studies

Six studies from seven countries have documented an echocardiography-based approach to screening asymptomatic school children for RHD (Anabwani and Bonhoeffer, 1996, Carapetis et al., 2008, Marijon et al., 2007, Paar et al., 2010, Bhaya et al., 2010, Beaton et al., 2012) (Table 1.2). These studies reported prevalence rates for definite or probable RHD from 2.7/1000 to 50/1000 school children with respective sample sizes ranging from 1059 to 5053 participants. The criteria used to assess the participants in these studies were based on the guidelines formulated by a working group convened and supported by the U.S. National Institutes of Health and the World Health Organization (Carapetis JP et al., 2006), except for Marijon, who developed a set of consensus guidelines among the participating centres and Anabwani who conducted the first echocardiography-based

screening study by colour-flow Doppler. The approach in all of these studies however, called for confirmation of the diagnosis of RHD by a cardiologist, who may not be available in a vast majority of African countries.

7.1.2 Previous Studies in South Africa

Previous studies in South Africa have been reviewed in more detail in Section 1.5.3. Briefly, there are four studies on the prevalence of RHD among school children in South Africa. Two studies conducted in schools on the outskirts of Johannesburg reported a prevalence per 1,000 of between 5 and 10 in 428 children (Pocock et al., 1968) and 6.9 in 12,050 children (McLaren et al., 1975). The remaining studies reported prevalence rates of 6.9 (n=1150) and 1.0 (n = 4408) from schools in Hout Bay, Cape Town (Bundred, 1986) and from Inanda, Durban (Maharaj et al., 1987). All of these studies employed cardiac auscultation in initial screening of the children and thus, the findings are likely to be an underestimate, given the consistent demonstration of the superiority of echocardiography over auscultation in detecting subclinical RHD (Carapetis et al., 2008, Marijon et al., 2008).

The paucity of epidemiological data from developing countries on the burden of streptococcal disease has been described (Carapetis et al., 2005b). Given that screening of asymptomatic school-age children is a useful strategy to identify new cases of RHD in regions of high prevalence (Carapetis et al., 2008), we conducted this echocardiography-based study of the prevalence of RHD in school children in the Vanguard community of Cape Town, given the greater yield of echocardiography over auscultation (Marijon et al., 2008). For this study, a new set of criteria was introduced which focused on the echocardiograph as a screening tool without the concomitant use of clinical examination in the screening phase (Zuhlke and Mayosi, 2009).

7.2 PATIENTS AND METHODS

The methods are described in detail in Section 2.4 on 'Rationalé and Design of the studies". As discussed, the evaluation procedures used in the previous school screening studies relied upon cardiologists; instead, we conducted our study according to echocardiographic protocols suitable for use by echocardiographic technicians within a setting such as South Africa (Zuhlke and Mayosi, 2009). RHD-positive participants were categorised following the screening procedure as having definite, probable or possible disease on screening echocardiograms according in to the criteria in Box 7.1. A cardiologist reviewed the first 798 echocardiograms to test for agreement with the echocardiography technologist.

Box 7.1. Definitions of definite, probable and possible RHD used in the Cape Town study as per the echocardiographic criteria published by Zuhlke et al. for use where there is no cardiologist (Zuhlke and Mayosi, 2009).

Definite RHD
Significant mitral stenosis (mean gradient: >4mmHg)
Significant structural and/or functional changes involving both mitral and aortic valves, i.e., multiple valve disease
Probable RHD
Significant structural and functional changes involving either mitral or aortic valves, i.e., single valve disease
Possible RHD
Isolated structural OR functional changes involving either mitral or aortic valve

7.3 RESULTS

Two thousand, seven hundred and twenty healthy (as per self-reporting) school children were examined from 23 government schools in the two school districts of Bonteheuwel and Langa. Pairwise kappa test showed fair agreement between the echocardiographic technician and the cardiologist (agreement, 93.11%; $\varkappa = 0.3$; SE = 0.0329).

Participants had a mean age of 12.2 years (range, 4 – 24 years) with all age categories well represented (Figure 7.1). The school districts did not differ with regard to gender, but

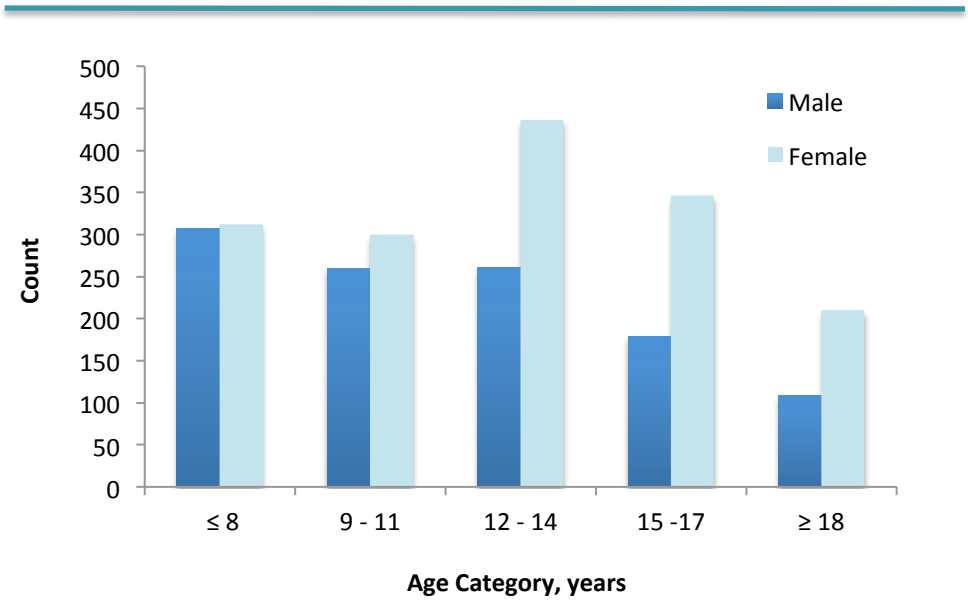

Figure 7.1. Age and Gender Distribution of participants from the Bonteheuwel and Langa School Districts, Cape Town, South Africa

learners from Bonteheuwel had a significantly lower mean age (mean difference = -1.814 years, 95% CI -2.12; -1.51). Older age groups tended to be female children ($p < 0.05$). Three hundred and nineteen participants (11.7%) were aged 18 years or older. Table 7.1 presents the baseline characteristics according to school district.

Table 7.1. Characteristics of the participants

	School District		
	Bonteheuwel (n = 1,303)	Langa (n = 1,417)	Combined (n = 2,720)
Baseline Characteristic			
Age: Mean (SD), y	11.2 (3.76)	13.1 (4.33)	12.2 (4.17)
Range	4 – 21	4 – 24	4 – 24
Median	11	13	12
Age breakdown, y			
≤ 8	355	264	619
9 - 11	302	258	560
12 -14	360	337	697
15 -17	233	292	525
≥ 18	53	266	319
Males, n (%)	555 (42.59)	561 (39.59)	1116 (41.03)
Cases of RHD, n			
Definite	5	11	16
Possible	182	162	344
Probable	23	39	62
Total	28	50	78

n, number; SD, standard deviation; y, years; CI, confidence interval; examined by echocardiography; pr, prevalence; CI, confidence interval

Using echocardiography, we detected RHD in 78 children overall, 16 of whom were classified as definite RHD and 62, as probable RHD as per our protocol (Table 7.2.),

Table 7.2. Prevalence rates of echocardiography-confirmed definite and probable RHD among asymptomatic school children

	School District		
	Bonteheuwel (n = 1,303)	Langa (n = 1,417)	Combined (n = 2,720)
Prevalence of RHD /1000 (95% CI)	21.5 (14.3 – 31.0)	35.3 (26.3 – 46.2)	28.7 (22.7 – 35.7)
Prevalence by gender			
Boys	16.2 (7.4 – 30.6)	32.1 (19.1 – 50.2)	24.2 (16.0 – 35.0)
Girls	25.4 (15.4 – 39.4)	37.4 (25.7 – 52.4)	31.8 (23.7 – 41.6)
Prevalence by Age (y) Strata			
≤ 8	5.63 (0.7 – 20.2)	11.36 (2.3 – 32.9)	8.08 (2.6 -18.7)
9 - 11	36.42 (18.3 – 64.2)	27.13 (11.0 – 55.1)	32.14 (19.2 – 50.3)
12 -14	25.0 (11.5 – 46.9)	29.67 (14.3 – 53.9)	27.26 (16.5 – 42.2)
15 -17	17.17 (4.7 – 43.4)	41.10 (21.4 – 70.7)	30.48 (17.5 – 49.0)
≥ 18	37.74 (4.6 – 129.8)	67.67 (40.6 – 104.8)	62.70 (38.7 – 95.2)
Children under 18 years of age			
Prevalence / 1,000	20.80 (13.6 – 30.3)	27.80 (19.1 – 39.0)	24.16 (18.4 – 31.1)
Estimated No. of Cases, n (95% CI)			
Vanguard School District	288 (256 – 323)	262 (232 – 295)	563 (518 – 611)
South Africa			353,581 (325,235 – 383,367)

n, number; CI, confidence interval; y, years; examined by echocardiography; pr, prevalence; CI, confidence interval.
**Number of cases was estimated by applying the observed prevalence in our sample to the whole population of under 18 years of age in the respective suburbs.*

which corresponds to a prevalence of 28.7 per 1,000 (95% CI, 22.7 – 35.7 per 1,000). Based on the 2003 census data, we estimate the number of prevalent cases to be 288 and 262 in Bonteheuwel and Langa school districts respectively, which translates to 350,000 cases nationally. The overall prevalence of RHD was higher in female children (31.8/1,000 vs 24.2/1,000 in males). Concerning age, a peak prevalence of 32.1/1,000 (95% CI 19.2 – 50.3/1,000) was observed in the 9 – 11 years old age group.

Of the 16 participants classified as having definite RHD, 11 (68.8%) were from the Langa school district. Those from Langa were significantly older with a mean of 15.5 years vs 9.8 years in Bonteheuwel (p=0.004) and more likely to be female (p=0.02). The respective prevalence rates for definite (95% CIs/1,000) are as follows: Bonteheuwel 3.8/1,000 (1.2 – 8.9), Langa 7.8/1,000 (3.88 – 13.85) giving a combined prevalence rate of 5.88/1,000 (3.37 – 9.54) children.

7.4 DISCUSSION

This is the first echocardiography-based investigation of the prevalence of RHD in asymptomatic school children in South Africa. Screening was conducted in two school districts of Cape Town by echocardiography technicians using WHO criteria modified for school-based screening without the need for interpretation by a cardiologist (Zuhlke and Mayosi, 2009). Thus, we demonstrate the value of screening performed primarily by a technologist.

The prevalence of RHD amongst school children in our population was found to be 28.7 per 1,000. School children in our cohort included a significant proportion above the age of 18 years; on excluding these participants from the analysis, 58 children out of 2401 had

definite or probable RHD, corresponding to a prevalence of 24.2 / 1,000 children under the age of 18 years. The prevalence increased four-fold from 0.8% among children aged 8 years and younger to 3.2% among those aged 9 – 11 years, which follows closely the expected pattern of a true reflection of prevalent cases in a population (Carapetis et al., 2005b).

Of the 16 participants classified as having definite RHD, 11 (68.8%) were from the Langa school district. Those from Langa were significantly older with a mean of 15.5 years vs 9.8 years in Bonteheuwel (p=0.004). This finding thus highlights the risk associated with a lower socio-economic status (Beaton et al., 2012). Also, females were more likely to have definite RHD (p=0.02), a finding which confirms those reported in other studies (Saxena et al., 2011, Marijon et al., 2009).

Figure 7.2 shows the relative prevalence (according to WHO criteria for definite RHD or probable RHD) across recent studies which used echocardiography for screening school children. The prevalence for Mozambique are according to the authors' revision of the original data using the WHO criteria (Marijon et al., 2009). In comparison, our results fall below the range of previously reported prevalence rates in similar populations in Tonga, 33.2/1,000 (Carapetis et al., 2008) and India, 50/1,000 (Bhaya et al., 2010). One reason for this may relate to the relative stable nature of the communities from where our participants were selected which, despite their lower socioeconomic status, have reasonable access to primary health care facilities for treatment of pharyngitis.

Earlier studies in South Africa reported prevalence rates of up to 7 per 1000 in school children from lower socioeconomic circumstances (Pocock et al., 1968, Maharaj et al., 1987, Bundred, 1986). We report a fourfold increase, a finding that is more likely to be

accurate given the superiority of screening by echocardiography and the lower likelihood of subjectivity in diagnosis.

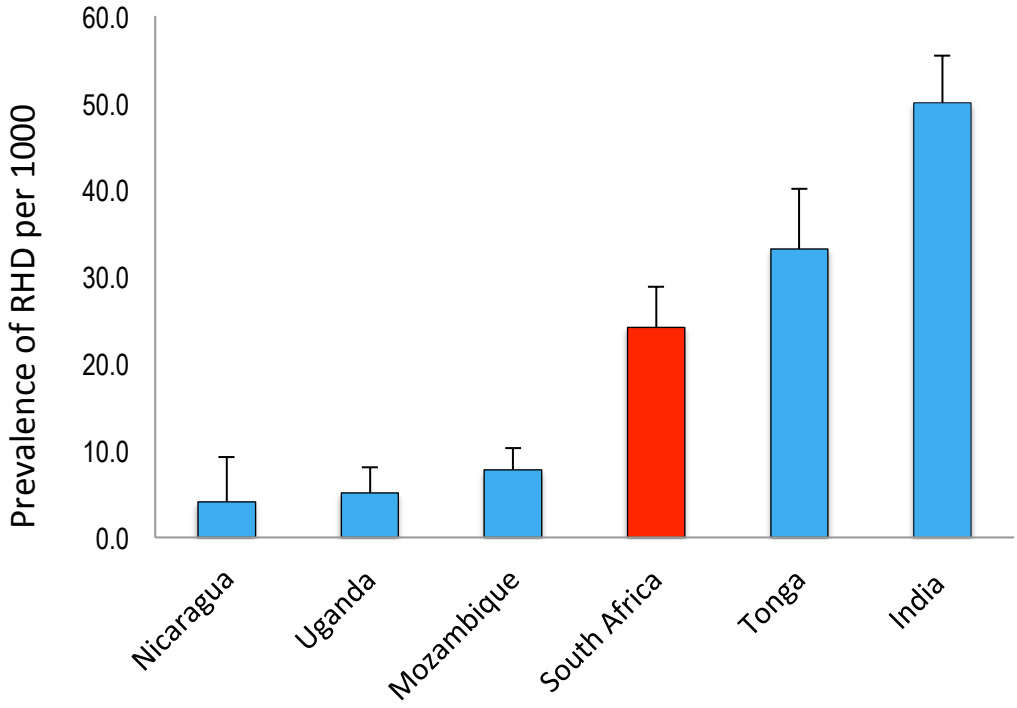

Figure 7.2. Prevalence of echocardiographically-confirmed RHD based on WHO or WHO modified criteria for Definite and Probable RHD in school children aged < 18 years.
bars indicate the superior limit of the 95% CIs.

This study has a number of strengths including that it was adequately powered given the good level of enrolment. A clustered sampling approach using the classes as the unit of randomisation proved successful with almost everyone approached agreeing to participate, thereby resulting in good representation in all age groups and across both school districts. Furthermore, consistency in procedures was optimal given that the echocardiography was performed almost exclusively by one of two echo technologists using one of two

echocardiography machines. A limitation of this work was that we did not use the World Heart Federation echocardiography criteria in evaluating our screening echocardiographic images, given that these were only recently introduced (Remenyi et al., 2012). In addition, amongst the higher grades, there was a skewing of gender towards female learners, thus giving rise to the potential of having missed male learners with asymptomatic disease.

In conclusion, this is the first screening study, to the best of our knowledge, that reports screening being conducted completely by an echo technologist. Given the dearth of cardiologists in many developing countries where RHD is prevalent, we strongly suggest that similar studies be conducted to confirm the feasibility of echo technologist-led screening programmes. This study emphasizes the increased risk of contracting RHD in marginalised peri-urban communities, thus lending support to the importance of adopting primary antibiotic prophylaxis strategies, especially in areas of high prevalence, in an effort to reduce the global burden of RHD (Karthikeyan and Mayosi, 2009).

8 CONCLUSION AND FUTURE DIRECTION

SUMMARY AND CONCLUSIONS
IMPLICATIONS FOR POLICY, PRACTICE AND FUTURE RESEARCH

This thesis has resulted in a series of five linked studies on the determinants and prevalence of RHD in Cape Town. The synopsis below reflects the novel contributions made by the various studies in addressing the original aims of the thesis. Implications for policy and practice, as well as recommendations for future research are presented.

8.1 ORIGINALITY / NOVEL INSIGHT

The first study in this thesis identified and summarized all studies reporting on the concordance of ARF amongst twin pairs, and is the first to provide a quantitative effect of the size of the genetic effect of ARF. The study provides strong justification for whole genome studies of the genetic basis of ARF and RHD.

This next study was the first investigation of pharyngeal carriage of β-haemolytic streptococci among school children in South Africa in more than 30 years. Carriage of group A streptococci in the Vanguard Community was found to be remarkably low overall. Nevertheless, participants enrolled from two communities comprising Vanguard differing with regard to socioeconomic status, and from a wide age range showed that rate of GAS carriage is significantly associated with socioeconomic factors prevalent in poorer communities. This finding also confirms reports elsewhere indicating an environmental influence in GAS pharyngeal carriage. In addition, this was the first study in South Africa, and only the second in Africa, to conduct molecular characterisation of *emm* strains associated with GAS carriage in Africa, showing that only around 46% of carriage strains

are included in the putative 30-valent vaccine currently under development.

Another noteworthy contribution of this thesis is the first prospective incidence study of acute GAS pharyngitis in 3- to 15- year old children with pharyngitis in South Africa. Younger children were observed to have twice the period prevalence and incidence rates of older children, confirming results from studies elsewhere.

Next, this thesis provided the first South African Clinical Prediction Rule for GAS pharyngitis developed using data from local communities. CPRs for GAS pharyngitis that had been developed abroa, including the diagnostic algorithm promoted by the WHO, were shown to be inadequate at predicting GAS pharyngitis amongst our children,.

Finally, this thesis provides the first estimate, using an echocardiography-based screening approach, of the burden of RHD in South African school children. In our setting, technologist-driven screening was shown to be feasible. There was a clear socio-economic gradient in the prevalence of severe RHD, resulting in a higher prevalence of definite RHD in the Langa community.

8.2 IMPLICATIONS FOR POLICY AND PRACTICE

This thesis emphasizes the need for primordial prevention of RHD through addressing the needs related to socioeconomic status such as adequate housing, overcrowding and impoverished living conditions.

Furthermore, access to adequate health care facilities where staff are trained in the implementation of guidelines, assisted by the newly-developed CPR, in order to recognise the clinical signs of GAS pharyngitis, in the absence of microbiological culture needs to be prioritized.

Finally, the high burden of RHD amongst asymptomatic school children lays the basis for studies of cost-effectiveness of screening in programmes to prevent ARF and RHD.

8.3 IMPLICATIONS FOR FUTURE RESEARCH

The evidence provided for genetic susceptibility for RHD provides justification for the launch of whole genome studies of ARF and RHD.

A follow-up study in those participants identified as GAS pharyngeal carriers would render valuable information on the persistence of strain types in our population. In particular, there is a need to monitor carriers in order to ascertain whether they switch *emm* types during episodes of active GAS pharyngeal infection.

There is a need to conduct *emm* strain typing on GAS isolates from patients presenting with pharyngitis at primary health care facilities in order to compare strains with those isolated from carriers. This information will provide valuable insight into vaccine development.

The CPR for pharyngitis developed during this thesis needs further validation, given the relatively small number of patients available at the time of this study.

The recent revision of the World Heart Federation echocardiographic criteria for screening requires the re-examination and scoring of the echocardiograms according to the new standard, which would allow comparison with other studies.

Finally, given that echocardiography-based school-screening has been proven to be feasible in our setting, there is a need to conduct a cost-effectiveness analysis of such screening in order to provide justification for adopting policy in this regard.

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10 APPENDICES

*10.1 **Appendix 1:** PRISMA Checklist*

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	

Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

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*10.2 **Appendix 2:** Consent and Assent Forms used in the Clinic-based Studies*

Parental Information Sheet:

The Molecular Epidemiology of *Streptococcus pyogenes* sore throat among Children in the Vanguard Community (Bonteheuwel/Langa), Cape Town, South Africa and The Development of a Clinical Prediction Rule for Streptococcal sore throat

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INTRODUCTION

Your child has been asked to participate in this research study because he or she has a sore throat. We are asking for your permission for your child to participate.

This form provides information about the study and the risks and benefits of being involved in this study. Please read it carefully and then ask the study doctors or nurse any questions that you may have.

Your child does not have to take part in the study. If you choose not to take part, your child will still be treated for his/ her sore throat. You will not be penalised for not participating in this study.

WHY ARE WE DOING THIS STUDY?

There are many causes of sore throat. Most often a sore throat is caused by a virus. A virus is a germ which can cause illnesses, for example colds and flu. Antibiotics do not work against viruses. Antibiotics should only be used when a doctor or clinical nurse believes that a patient's illness is caused by a bacterium, which is a different kind of germ which can be killed by antibiotics.

If antibiotics are used too often and incorrectly, bacteria become resistant to the antibiotics. This means that antibiotics no longer work against them and so in future, we may be unable to treat infections caused by these bacteria.

A particular type of bacterium called *Streptococcus pyogenes* can cause sore throat. As a complication of this type of sore throat, a small percentage of children can later develop disease of the heart valves

(Rheumatic heart disease) or kidney disease. Antibiotics can prevent children from developing these complications.

When a child has a sore throat, the doctor or clinical nurse has to decide whether the likely cause is a virus or a streptococcal infection, in order to decide whether or not antibiotics should be prescribed. There are certain symptoms and signs which may help the doctor or nurse to decide what is the most likely cause of a particular sore throat. Doctors and nurses ask the patient and their caregiver questions, and examine the child to look for these signs. In previous studies, these symptoms and signs have been combined into "Clinical prediction rules"- a scoring system which helps the doctor or nurse to decide whether or not a sore throat is due to streptococcal infection.

These scoring systems have been studied in other parts of the world to see how good they are at identifying children who have streptococcal sore throat. Their performance in South African children has not previously been studied.

In this study, we will test the performance of existing prediction rules in South African children, and develop a clinical prediction rule for use in South African children.
This information will be very helpful to healthcare workers who can use this to make decisions about the need for an antibiotic.

There are many different genetic types of *Streptococcus pyogenes* and it is not known which types of this bacterium are most common in your area. In the study, we will take throat swabs which will be sent to a laboratory in the United States to examine the genetic structure of the bacteria. The genes of the bacteria, and not your child, will be investigated.

It is hoped that if we know which genetic types are most common, it will help researchers to produce an effective vaccine against *S pyogenes*. A vaccine is a substance that is used to protect a healthy person from developing an infection.

IF WE PARTICIPATE, WHAT WILL BE EXPECTED OF US?

If you agree to participate in the study, the study nurse or doctor will ask you questions about your child and their illness.

After the questions, your child's neck and throat will be examined.

A throat swab will then be performed. Using a long plastic stick with cotton wool at the end (much like an earbud) a sample of the mucous or pus from the back of the throat will be taken. This sample will be sent to a laboratory where we will look for the presence of *Streptococcus pyogenes*.

Afterward, the clinic doctor or nurse will examine and treat your child as usual.

WHAT WILL HAPPEN TO MY CHILD'S THROAT SWAB AT THE END OF THE STUDY?

Throat swabs collected from your child during this study will be labelled with a unique code. It will not be possible to determine your child's identity from this information. Should your child's swab contain bacteria, the sample will be stored. We may study these bacteria further at a later date. You will not be informed of any results of these studies, nor benefit financially from any medicines or vaccines which are produced by these studies.

Any future research with your child's samples will be reviewed by the University of Cape Town and the University of Tennessee Institutional Review Board before the research can begin. Your permission is required for these later studies.

If you do not agree to the storage and later investigations of the bacteria, you can still be involved in the rest of the study procedures.

WHAT ARE THE RISKS OF BEING INVOLVED IN THE STUDY?

Taking the throat swab can be uncomfortable, especially for younger children. Touching the back of the throat may make your child feel nauseous for a little while. We will try to make the procedure as quick and comfortable as possible.

WHAT ARE THE BENEFITS OF TAKING PART IN THIS STUDY?

The results of the laboratory diagnosis of your child's swab will be made available to the clinic staff to be added to your child's folder. Also, you will be helping us to produce a guideline to assist nurses and primary care doctors in deciding which people with sore throats need antibiotics. Such a rule would be of benefit to future patients with sore throat as it will help doctors and nurses treat patients appropriately. You will also possibly be helping researchers to produce a vaccine that is effective in our setting and which may one day help in the prevention of the complications of sore throat.

HAS THIS STUDY BEEN APPROVED?

This study is being performed by investigators from the Department of Medicine and the Department of Medical Microbiology at Groote Schuur Hospital.

The study has been approved by the Human Research Ethics Committee of the University of Cape Town. If you have any problem with this study, please contact this committee at 021 406 6338.

YOUR RIGHTS AS A PARTICIPANT IN THIS STUDY

You have the right to refuse to participate in this trial. Your child will still be treated in the same way if you do not participate. You have the right to withdraw from the study at any time.

CONFIDENTIALITY

All information collected during the course of this study will be kept securely and confidentially. Reports about the study and results that may be published in scientific journals will not include any information which identifies you or your child personally.

If the investigators have any questions about any of your child's medical information which is important for this study, we may need to find the clinic folder to gather information. This information will be treated as confidential.

INFORMED CONSENT: The Molecular Epidemiology of *Streptococcus pyogenes* pharyngitis Among Children in the Vanguard Community (Bonteheuwel/Langa), Cape Town, South Africa and The Development of a Locally Applicable Clinical Prediction Rule for Streptococcal Pharyngitis

- I hereby confirm that I have been informed about the purpose, benefits and risks of allowing my child to participate in this study.
- I am aware that some information regarding my child's health will be used anonymously by the investigators and researchers.
- I have been informed that participation is voluntary.
- I have had an opportunity to ask questions and these have been answered
- I have read and understood the contents of this document.

I agree to the storage and later investigations of bacterial specimens obtained from my child's throat.
YES / NO

Participant:

_____

Parent or legal guardian::

_____

Printed name Signature Date

Study Doctor or Research Nurse:

_____

Printed Name Signature Date

Witness:

_____

Printed Name Signature Date

Assent Form

The Molecular Epidemiology of *Streptococcus pyogenes* pharyngitis Among Children in the Vanguard Community (Bonteheuwel/Langa), Cape Town, South Africa And The Development of a Clinical Prediction Rule for Streptococcal Pharyngitis

We are doctors and nurses from Groote Schuur Hospital and we are doing a study of sore throat in children. Sore throat can be caused by many kinds of germs.

We would like to ask you and your parent / legal guardian a few questions about your sore throat. After that, the nurse will ask to look inside your mouth at your throat and feel your neck for swollen glands.

To find out if you have a germ in your throat, we will use a long soft stick (like an earbud) and lightly touch the back of your throat. This will not hurt at all and is very quick, but may be a little uncomfortable. This stick will then be sent away to test for a germ that causes sore throat.

If you allow us to examine you, you will be helping doctors understand sore throat better. Your information will help doctors and nurses to learn how to treat sore throat in children.

You are allowed to say that you don't want to be in the study. Nobody will be angry with you if you say no. The clinic nurses will still treat your sore throat if you don't want to be in the study.

Before you decide, you can ask us questions. If you want to be in the study, you must write your name on this sheet of paper. This means that you are happy to be involved in the study.

Participant:

_____

Printed name Signature Date

Study doctor or research nurse:

_____

Printed name Signature Date

Witness:

_____

Printed name Signature Date

*10.3 **Appendix 3:** Case Report Form used in the Clinic-based Studies*

CASE REPORT FORM

Screening Number: | | | | |

I. General Information

1	Enrolment Date	d d m m y y	2	Clinic name	*VAN / NET / LAN*
3	Clinic folder #				
4	Date of birth	d d m m y y	5	Age	
6	Gender	m f	7	Race	Black White Ind Col Other
8	Place of residence	**B L Oth**			

Has the patient used antibiotics within the last 30 days? Y N
If yes, do not continue

Informed consent obtained Y N
If NO, do not continue

II. Pharyngitis

Participant Number: | | | | |

Mark the signs and symptoms obtained from history and examination of the child:

		Y	N			Y	N
1	Cough	1	0	2	Rhinorrhoea	1	0
3	Hoarseness	1	0	4	Temperature > 38°C	1	0
5	Tonsillar erythema	1	0	6	Tonsillar swelling	1	0
7	Exudate on the pharynx	1	0	8	Exudate on the tonsils	1	0
9	Oropharyngeal candidiasis	1	0	10	Tender anterior cervical node	1	0
11	Anterior cervical node > 1.5cm in diameter	1	0	12	Rash	1	0

Time of swab collection _____

Person completing form (Name) _____

(Signature) _____

*10.4 **Appendix 4:** Standard Operating Protocol: Throat Swab Collection*

Collection of specimens.

All throat swab specimens will be collected by study staff using a standard procedure. For each throat specimen, a single sterile individual cotton swab will be slowly swiped across one tonsil or tonsillar fossa, then across the posterior pharynx, and finally across the opposite tonsil or tonsillar fossa. The collector will avoid touching the tongue or the mouth with the swab. Swabs with liquid media tips will be used (Culturette or equivalent). These swabs will be returned to their sheaths following swabbing. The sheath contains a transport medium at the tip for the swab.

Transport of specimens.

Swabs collected at the clinical sites will be transported to the National Health Laboratory Service (NHLS) microbiology laboratory at Groote Schuur Hospital for incubation. Specimens will be kept in an insulated bag for specimens directly to the Groote Schuur Hospital laboratory where the culture will be performed. In the unlikely event of the study nurse/ coordinator being unable to transport the specimens, another member of the research team will take on that responsibility.

Handling of specimens.

Swabs will be received by the microbiology laboratory and logged in according to routine procedures. Specimens received as appropriately labelled swabs will be plated onto 4% sheep blood agar plates in a standard fashion. Every effort will be made to place specimens in an incubator no later than 4 hours from the time of collection. Time of collection and time of plating will be recorded. Plates will be inverted and incubated anaerobically at 35°C. After 18 to 24 hours, the plates will be read by the microbiology technician, under the supervision of one of the investigators, Dr Andrew Whitelaw. All cultures of beta-haemolytic colonies will be further identified by Gram stain, catalase, and serogrouping, as appropriate. A single colony will be picked off with a sterile wire loop, and sub-cultured for purity. Pure colonies of beta-haemolytic streptococci identified as group A, C or G will be removed from the plates in a sterile fashion and placed in trypticase soy broth with glycerol for storage. Triplicate vials will be made for each isolate. The cryovials will be labelled with identifier numbers and placed in the 70°C freezer. The report containing the microbiologic data indicating the presence or absence of beta haemolytic streptococci (group A, C or G in particular) and the storage identification number will be affixed to the case report form.

*10.5 **Appendix 5:** Consent Form used in the School Screening Study*

Prevalence of RHD in Cape Town

Name of Research Study: Study of the Prevalence of Rheumatic Heart Disease in school children in the Vanguard Population in Cape Town, South Africa

Principal Investigator: Bongani M. Mayosi, D.Phil. Groote Schuur Hospital
Tel: 27-21-406-6200 Email: bongani.mayosi@uct.ac.za

Consent Form for Parent(s) / Guardian(s) of Children

Introduction
This consent form contains information about the research study named above. So we can be sure that you and your child are informed about being in this research study, we are asking you to read (or have read to you) this Consent Form. You will also be asked to sign it (or place your thumb print in front of a witness). You must sign this parental consent form and return it to the research staff before your daughter/son can take part in the research. If your child is 8 years old or older, he/she will also be informed about the study and asked to agree to take part. We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Why is the Study Being Done?
A bacterium called Group A Streptococcus or "strep" is commonly found in the nose and throat of healthy adults and children, and can cause a wide variety of illnesses including sore throat, skin sores, and blood stream infections. These illnesses must be diagnosed and treated with the right medicine. If the streptococcus bacterium infects another part of the body one or more times, children may get Rheumatic heart disease (RHD). The valves of the heart can be affected which can lead to permanent damage. Some children may have RHD and not know it, because it may only cause problems later in life.

This research is being done to study RHD. The main reason for the study is to see how many children between 5 – 17 years of age have RHD. We also want to find out how well the usual methods for detecting RHD are working. We hope that this study will collect information that will help to develop and bring vaccines that may prevent infections due to "strep" to Cape Town and other parts of the world in the future.

What is involved in the Study?
If you agree to have your child participate in this research, we will ask some general questions about your family and your child. Thereafter, your child will be examined and we will perform a throat swab. Using a long plastic stick with cotton wool at the end (much like an earbud), we will rub over the skin at the back of the throat. The swab will then be sent to a laboratory where we will look for the presence of a bacteria called Streptococcus pyogenes. Afterward, the researcher will do a sound wave test of your child's heart, called echocardiogram. This will tell us if your child might have a heart problem. If we find that your child already has heart disease or may be at risk to develop RHD, you will be referred to Red Cross Children's Hospital for follow-up and treatment. If we are not sure if the heart problem is RHD, we may ask your child to come back for a new echocardiogram in 6 months. You will be asked to give permission for your child to continue in this research. If you agree, we will ask you to sign another consent form before your child is examined.

About 3,000 children from Bonteheuwel and Langa will take part in this research. The entire study will take about 2 years.

What will happen to my child's results at the end of the study?

All information relating to your child during this study will be labeled with a unique code. It will not be possible to determine your child's identity from this information. Should your child's swab contain bacteria, the sample will be stored. We may study these bacteria further at a later date.
Any future research with your child's samples will be reviewed by the University of Cape Town's Institutional Review Board before the research can begin. Your permission is required for these later studies.

If you do not agree to the storage and later investigations of the bacteria, you can still be involved in the rest of the study procedures.

What are the Possible Risks and Benefits of the Study?
There is very little risk involved in taking part in this research. The tests we will do will not hurt or pose any risk to your child. Taking the throat swab may be uncomfortable, especially for younger children since touching the back of the throat may make your child feel nauseous for a little while. We will try to make the procedure as quick and comfortable as possible.

Your child may benefit from taking part in the research by referral for treatment if we detect a problem with his or her heart.

What if I or My Child decides not to be in the Research?
You and your child are free to refuse to be in this research study. No record will be kept of this, nor will it affect the health care your child would normally receive.

Has this study been approved?
This study is being performed by investigators from the Department of Medicine at Red Cross Children's Hospital and Groote Schuur Hospital. The study has been approved by the Human Research Ethics Committee of the University of Cape Town. If you have any problem with this study, please contact this committee at 021 406 6338.

What about Confidentiality?
You must sign this parental consent form and return it to the research staff before your daughter/son can take part in the research. We will protect information about your daughter/son and her/his taking part in this research to the best of our ability. Your child will be identified by a code on all study records. Personal information from his/her records will not be released without your written permission. If the results of this research are published, your child's name will not be shown. However, the staff of Red Cross Children's and Groote Schuur Hospitals may sometimes look at records of those people who take part in the research study.

Will we be Paid for Being in the Study?
You or your daughter/son will not be paid for being in the research.

What are the Costs?
There will be no costs to you or your medical aid for procedures that are part of this study.

How Long Will My Child be in the Study?
The examinations will take about 1 hour at the most.

Can My Child Leave the Research Study?
Your child may leave the research study at any time without losing the benefits of any of the regular care he or she would normally receive.

Also, your daughter/son may be asked to leave the research if

- the research doctor/clinic staff or you feel it is best for her/him, or
- the research is stopped.

What are My Child's Rights as a Study Participant?
Taking part in this study is voluntary. You may choose not to let your child take part in the study, or you or your child may decide to leave the study early. This will not cause any problems with your child's regular medical care.

If you have any questions about your daughter/son's rights while taking part in the research you may contact the Human Research Ethics Committee of the University of Cape Town on (021) 406-6338.

What if My Daughter/Son has a Problem?
If your daughter/son has a problem that you or she/he thinks might be related to taking part in this research, please call our office on 021-406-6361.

If your daughter/son is sick or has a health problem due to her/his participation in this research, you will not have to pay for visits to see the research doctor/clinic staff.

VOLUNTEER AGREEMENT

Name of Research Study: Study of the Prevalence of Rheumatic Heart Disease in school children in the Vanguard Population in Cape Town, South Africa

1. STATEMENT BY PARENT/GUARDIAN AGREEING FOR HIS/HER CHILD TO PARTICIPATE IN THIS STUDY

I have read this informed consent document describing the benefits, risks and procedures for the research study titled "Study of the Prevalence of Rheumatic Heart Disease in school children in the Vanguard Population in Cape Town, South Africa" or it was read to me. I freely and voluntarily choose to participate/ to allow my son/ daughter to participate in the study.

Name of participant: _____
Name of child

_____ _____
Date Signature or thumbprint of parent/ guardian

2. IF THE PARENT/GUARDIAN CANNOT READ THE FORM THEMSELVES, A WITNESS MUST SIGN HERE:

I was present while the informed consent document with benefits, risks and procedures were read to the parent/guardian and the participant. The parent/guardian has freely and voluntarily agreed to allow her/his daughter/son to take part in the research.

_____ _____
Date Signature of witness

*10.6 **Appendix 6: :** Assent Form used in the School Screening Study*

Assent Form for Children 8 years and older

Name of Research Study: Study of the Prevalence of Rheumatic Heart Disease in school children in the Vanguard Population in Cape Town, South Africa

Principal Investigator: Bongani M. Mayosi, D.Phil. Groote Schuur Hospital
Tel: 27-21-406-6200 Email: bongani.mayosi@uct.ac.za

We are doctors and nurses from Groote Schuur Hospital and we are doing a study of hearts in children. Some heart diseases are caused by a specific type of germ living in the throats of children.

We would like to ask you a few questions about your health. After that, the nurse will examine your skin and joints. This will not hurt at all and is very quick.

We also want to examine your throat to make sure that you do not have any germs living there. We will use a long soft stick (like an earbud) and lightly touch the back of your throat. This will not hurt at all and is very quick, but may be a little uncomfortable. This stick will then be sent away to test for the germ that causes sore throat.

A special machine will be used to examine your heart. This will not hurt at all and is very quick.

If you allow us to examine you, you will be helping doctors to know how many healthy hearts and throats are present in the community.

You are allowed to say that you don't want to be in the study. Nobody will be angry with you if you say no.

Before you decide, you can ask us questions. If you want to be in the study, you must write your name on this sheet of paper. This means that you are happy to be involved in the study.

Participant:

_____

Printed name Signature Date

Study doctor or research nurse:

_____

Printed name Signature Date

Witness:

_____

Printed name Signature Date

*10.7 **Appendix 7:** Case Report Form used in the School Screening Study*

CASE RECORD FORM

RHEUMATIC FEVER / RHEUMATIC HEART DISEASE
SCREENING

PARTICIPANT NUMBER: _____

DIAGNOSTIC CATEGORY: (CIRCLE ONE ONLY) To be completed at end of today's examination

1st Attack ARF	= 01
Recurrence of ARF without RHD	= 02
Recurrence of ARF with RHD	= 03
Chorea	= 04
Insidious onset of rheumatic carditis	= 05
Known diagnosis of Chronic Rheumatic Heart Disease	= 06
School screening – normal	= 07
School screening – possible rheumatic process	= 08
School screening – probable rheumatic process	= 09
School screening - 1st diagnosis of Chronic RHD	= 10
School screening – Other (Specify _____)	= 11
Family screening - normal	= 12
Family screening - abnormal	= 13

MODE OF DIAGNOSIS:
- ☐ School screening
- ☐ Family screening
- ☐ Health care facility (Symptomatic)

DATE: _____

SIGNED: _____

DESIGNATION: _____

PART I: BASIC DATA

PARTICIPANT NUMBER: _____

DATE OF BIRTH: _____ *dd/mm/yyyy* *eg: 16/05/1991*

SEX: _____ (0 = unknown; 1 = male, 2 = female)

SUBURB: _____

ETHNICITY:
- ☐ Asian
- ☐ Black
- ☐ Coloured
- ☐ White
- ☐ Foreign Country: _____

SCHOOL NAME: _____

PART II: PAST MEDICAL HISTORY (ALL PERSONS SCREENED)

PRE-EXISTING DIAGNOSIS: 1. _____ 2. 3.

MONTH OF DIAGNOSIS: _____ MMM/YYYY *eg: JAN/1991, 0 = unknown*

DATE OF ADMISSION: _____ DD/MM/YYYY *eg: 16/05/1991, 0 = unknown*

DATE OF DISCHARGE: _____ DD/MM/YYYY *eg: 16/05/1991, 0 = unknown*

SYMPTOM TIME: _____ Days prior to presentation

THERAPY PRIOR TO PRESENTATION / ADMISSION: _____ 0 = unknown, A = nil, 1 = Aspirin, 2 = Paracet, 3 = other anti inflamm

Signed:_____ **Date**: **I__I__I / I__I__I / I 2 I 0 I 0 I__I**

Person completing section dd mm yyyy

PART III. AUSCULTATION / SKIN EXAMINATION

PART IIIA. CONSENT AND ANTHROPOMETRIC MEASUREMENTS

1. Participant Number	**I 1 I-I__I__I__I__I**
2. Initials**:**	**I__I__I / I__I__I**
3. Site:	Name_____
4. Site	Number **I__I__I**
5. Date of Enrolment:	**I__I__I / I__I__I / I 2 I 0 I 0 I__I** dd mm yyyy
6**.** Informed consent obtained?	☐ Yes ☐ No **IF NO DO NOT CONTINUE**
If no; why not	_____
7. Height I__I__I__I.I__I	8. Weight I__I__I__I.I__I 9. AGE I__I__I

PART IV. ECHO SCREENING DATA COLLECTION FORM

Participant number: **I 1 I-I__I__I__I__I**

Initials: **I__I__I / I__I__I**

Site: **Name _____** **Number I__I__I**

Date of enrolment: **I__I__I / I__I__I / I 2 I 0 I 0 I__I**

Step 1: PLAX 2D

Mitral valve:
- Normal ☐
- Abnormal ☐ →
  - Elbow deformity of the amvl ☐
  - Thickened amvl ☐
  - Thickened pmvl ☐
  - Elongation of amv chordae ☐
  - Tethered pmvl ☐
- Not sure ☐

Measured thickness of amvl at margin _____mm
Midleaflet _____mm
Measured thickness of pmvl at margin _____mm
Midleaflet _____mm

Nodules: ☐ **Yes** ☐ **No**

Aortic valve:
- Normal ☐
- Abnormal ☐ →
  - Thickened right coronorary cusp ☐
  - Thickened non-coronary cusp ☐
  - Thickened ring ☐
  - Thickened but not typically rheumatic ☐
- Not sure ☐

Nodules: ☐ **Yes** ☐ **No**

Step 2: PLAX M-MODE

Measurements:

At mitral valve tips:
- IVSd = ___
- LVIDd = ___
- LVPWd ___
- IVSs ___
- LVIDs ___
- LVPWs = ___
- EF = ___
- %FS = ___
- RVIDd = ___
- RVIDs = ___

At aortic cusp:
- Ao (diam) = ___
- LA (diam) = ___

Step 3: PLAX COLOR DOPPLER

Mitral valve: Normal ☐
Regurgitation ☐ → Jet direction _____
Pan-systolic ☐
Measure jet __ __ cm
Not sure ☐

Aortic valve: Normal ☐
Regurgitation ☐ → Jet direction _____
Measure jet : length _____cm
Measure jet : width _____cm
Not sure ☐

Step 4: PSAX 2D

Mitral valve: Normal ☐
Abnormal ☐ → Regurgitation ☐ → _____
Stenotic ☐ → _____
Not sure ☐
If stenotic or not sure must have PW Doppler

Aortic valve: Normal ☐
Abnormal ☐ → Thickened right coronorary cusp ☐
Thickened non-coronary cusp ☐
Thickened ring ☐
Thickened but not typically rheumatic ☐
Not sure ☐

Step 5: PSAX COLOR DOPPLER

Mitral valve: Normal ☐
Regurgitation ☐
Not sure ☐

Aortic valve: Normal ☐
Regurgitation ☐
Not sure ☐

Step 6: APICAL 2D

Mitral valve: Normal ☐
Abnormal ☐ → Elbow deformity of the amvl ☐
Thickened amvl ☐
Thickened pmvl ☐
Elongation of amv chordae ☐
Tethered pmvl ☐
Not sure ☐

Measured thickness of amvl at margin _____mm
Midleaflet _____mm
Measured thickness of pmvl at margin _____mm
Midleaflet _____mm

Aortic valve: Normal ☐
Abnormal ☐ → Thickened right coronorary cusp ☐
Thickened non-coronary cusp ☐
Thickened ring ☐
Thickened but not typically rheumatic ☐

Not sure ☐

Step 7: APICAL COLOR DOPPLER

Mitral valve: Normal ☐

Regurgitation →
Good spectral envelope? Y N
Jet reaches back atrial wall? Y N
Measure Jet (cm)
Jet direction

Central	Medial
lateral	eccentric

Not sure ☐

Aortic valve: Normal ☐

Regurgitation →
Good spectral envelope? Y N
Jet direction

Central	Medial
lateral	eccentric

Measure Jet (cm) length _____
Measure Jet (cm) width _____
Measure Jet (m/s) PHT _____

Not sure ☐

Step 8: APICAL PW/CW DOPPLER

MV pressure half-time and MVA: _____seconds = MVA _____ cm^2
☐ Not done

If MS suspected:…… MV mean pressure gradient: _____mmHg
☐ Not done

PW/CW Doppler of the AV: _____m/s
☐ Not done

Step 9: Other abnormalities:

Step 10: summary

ECHOCARDIOGRAPHIC FEATURES OF RHD

Yes ☐ *If yes, continue below…* No ☐

☐ Definite RHD: Significant MS (Mean Grad: >4mmHg)
Significant structural and functional changes involving both mitral AND aortic valves OR multiple valve disease

☐ Probable RHD: Significant structural AND functional changes involving either mitral OR aortic valves
Single valve disease

☐ Possible RHD: Isolated structural OR functional changes involving either mitral OR aortic valves

Definitions

Significant structural changes:	Significant functional changes:
Thickness of mitral and aortic leaflets greater than 4mm	Significant mitral regurgitation:
Increased echogenicity of submitral structures	*defined as a mitral regurgitant jet at least 1 cm. from the coaptation point of the valve leaflets, seen in two planes, high velocity (mosaic pattern) and persisting throughout systole. Additional changes that may be present include multiple regurgitant jets and/or a posterolaterally-directed jet*
Rheumatic nodules giving a beaded appearance	
Prolapse of mitral, aortic or tricuspid valves	
Reduced mobility of leaflets	
Chordal tears	
Elbow or dog leg deformity of the anterior mitral valve leaflet.	Significant aortic regurgitation:
Fixed or markedly restricted motion of the posterior mitral leaflet	*defined as an aortic regurgitant jet at least 1 cm from the coaptation point of the valve leaflets, of high velocity (mosaic pattern) and seen in two planes*

Signed:_____ **Date**: **I__I__I / I__I__I / I 2 I 0 I 0 I__I**
Person completing section dd mm yyyy

THROAT SWAB FORM

Participant Number: | | | | | |

I. General Information

1	Enrolment Date		2	School	(Abbr)
3	Date of birth		4	Age	
5	Gender	m f	6	Race	Black White Ind Col Other
7	Place of residence	**B L Oth**			

Has the participant used antibiotics within the last 30 days? **If yes, do not continue** Y N

II. Clinical Examination

Mark the signs and symptoms obtained from history and examination of the child:

		Y	N			Y	N
1	Cough	1	0	2	Rhinorrhoea	1	0
3	Hoarseness	1	0	4	Temperature > 38°C	1	0
5	Tonsillar erythema	1	0	6	Tonsillar swelling	1	0
7	Exudate on the pharynx	1	0	8	Exudate on the tonsils	1	0
9	Oropharyngeal candidiasis	1	0	10	Tender anterior cervical node	1	0
11	Anterior cervical node > 1.5cm in diameter	1	0	12	Rash	1	0

Time of swab collection _____

Person completing form (Name) _____

(Signature) _____

10.8 ***Appendix 8:*** *emm Typing Protocol*

Genomic DNA Extraction

Isolates were subcultured from frozen stocks onto blood agar and incubated in presence of 5% CO_2 for 24 hours. Single colonies were suspended in 1 ml of autoclaved water in Eppendorf tubes (Eppendorf, Germany). After emulsifying the colonies, the bacteria were pelleted using a microfuge for 1 minute at 16500 x *g* (Eppendorf, Germany). The supernatant was discarded and genomic DNA was extracted from the bacterial pellet using an InstaGene matrix DNA extraction kit (Bio-Rad, France) as recommended by the manufacturer. Using a 1000 µl pipette tip, as instructed, InstaGene matrix was added to the pellet and incubated for 30 minutes at 56°C (Kendro, German). After incubation, the tubes were vortexed at level 8 on a Vortex-2 Genie (Scientific Industries, USA) for 10 seconds and placed on a heating block (Techne LTD, USA) at 100°C for 8 minutes. The Eppendorf tubes were then vortexed again at level 8 for 10 seconds followed by centrifugation in a microfuge at 16500 x *g* for 3 minutes. The supernatant, containing the extracted DNA, was used as a template for PCR amplification.

Polymerase Chain Reaction

The emm gene was selectively amplified using PCR according to the CDC protocol. This molecular method selectively amplifies a target gene or a portion of a gene. The amplification is achieved by the use of sequence-specific oligonucleotides, often referred to as primers. Primers work in pairs by binding on either strand of double-stranded DNA. The primers used in emm typing anneal to a conserved sequence internal to the emm gene. The primers (Table) used in this assay were synthesized at the Department of Molecular and Cell Biology, University of Cape Town, South Africa. Polymerase chain reaction amplification of DNA is achieved by the work of an enzyme called DNA polymerase I (Taq Polymerase) isolated from the bacterium Thermus aquaticus. Taq Polymerase has the ability to withstand high temperatures during PCR cycles.

To achieve a successful amplification of the emm gene, a combination of reagents were used at specific concentrations recommended by the CDC. PCR reagents comprised 1X buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 70 picomol/µl of the forward and reverse primers and 1.5U of SuperTherm Taq (JMR, Holdings, London UK) then brought to a final volume of 50 µl with DNAse-free water.

Table. Primers used for *emm* Typing

Target gene	Primer	Primer sequence
***emm* gene**	Primer 1 (forward)	TAT T(C/G) GCT TAG AAA ATT AA
	Primer 2 (reverse)	GCA AGT TCT TCA GCT TGT TT
	Emmseq2	TAT TCG CTT AGA AAA TTA AAA ACA GG

Cycling conditions for amplification of the emm gene involves the three basic principle amplification steps during PCR, namely; denaturation, annealing and elongation. Cycling conditions for emm typing, as recommended by the CDC, begins with initial denaturation at 94ºC for a minute followed by 30 cycles of denaturation at 94ºC for 15 seconds, annealing at 46.5ºC for 30 seconds and extension at 72ºC for 75 seconds. Additional 20 cycles follow with denaturation at 94ºC, annealing at 46.5ºC for 30 seconds, extension at 72ºC for 75 seconds with a 10 second increment for each of the subsequent 19 cycles. Final extension was carried out at 72ºC for 10 minutes.

Agarose Gel Electrophoresis

Agarose gel electrophoresis is a technique used to separate DNA fragments based on their sizes when subjected to an electrical current. DNA has a negative charge due to the phosphate groups on its sugar-phosphate backbone and thus migrates to the positive end of the agarose gel when an electric current is passed through the gel. The percentage of agarose within a gel determines the pore size; hence different percentages of gels are prepared based on the size of DNA fragments to be separated. Larger DNA fragments migrate slower through an agarose gel than smaller fragments.

Agarose gel electrophoresis was used to separate the DNA fragments following PCR. As the expected sizes of emm amplicons range from 750 bp to 1400 bp, a 2% agarose gel was used to separate the PCR products. Agarose (SeaKem® LE Agarose, Lanza, USA) was dissolved in 1X TAE buffer (Appendix A) and ethidium bromide (10 ng/µl), which intercalates between base pairs of DNA and fluoresces under ultraviolet light. Eight microliters of the PCR product was loaded into each well, with 2 µl of loading dye. The fragments were separated by passing an electric current through the agarose for 3 hour of 60V to allow the negatively charged DNA fragments to migrate towards the positive electrode. The rate of migration is inversely proportional to the size of the fragments. A molecular marker (HyperLadder IV, Bioline, UK) was included to enable estimation of emm amplicon sizes.

DNA Purification

The DNA sequencing assay is very sensitive and requires a purification step to minimise inhibition by PCR reagents within the sample. The MinElute PCR purification kit (Qiagen, Valencia, CA, USA) incorporates spin-column technology using silica-gel membranes with selective binding properties to purify DNA directly from PCR products.

Five volumes of PB buffer was added to one volume of PCR product and mixed as per manufacturer's instructions. To achieve a pH of 5.0 as recommended, 10 µl of 3 M sodium acetate was added and mixed. The mixture was then added to 2 ml MinElute columns and centrifuged in a microfuge (Eppendorf, German) for 1 minute at 17900 x g. The flow through products were discarded and 750 µl of Buffer PE was added to the column which was again centrifuged for a minute at 17900 x g. Again, the flow through product was discarded and the column was centrifuged for a minute at 17900 x g. MinElute columns were then transferred to sterile 1.5 ml Eppendorf tube and the DNA was eluted following the addition of 10 µl EB buffer to the centre of the column membranes and centrifuged for a minute at 17900 x g. Purified DNA products were stored at 4ºC.

Sequencing and Assigning* emm *Types

Sequencing of purified DNA was done using the ABI Prism® BigDyeTM Terminator Cycle Sequencing kit (Applied Biosystems, USA) at Stellenbosch University, South Africa. The DNA concentration was adjusted to 10 ng/µl for amplicon less than 1000-bp and 20 ng/µl for amplicons more than 1000-bp as recommended by the sequencing facility at the Stellenbosch University, South Africa. Primer emmseq2 (Table), recommended by the CDC, was prepared to 1.1 ng/µl and used for sequencing reaction. Sequences generated were analysed using BioEdit v7.0.9 (Ibis Biosciences, USA). The sequences were submitted electronically to the S. pyogenes emm sequence database centre at the CDC which assigned all the emm types and subtypes.

*10.9 **Appendix 9:** Search Strategy For Twin Studies of ARF and RHD*

Search performed on Pubmed	
1	RHEUMATIC FEVER OR RHEUMATIC HEART
2	FAMIL* OR TWIN OR ADOPTION
3	#1 AND #2
Search performed on EMBASE (www.embase.com)	
1	'RHEUMATIC FEVER'/SYN OR 'RHEUMATIC HEART'
2	FAMIL* OR 'TWIN'/SYN OR 'ADOPTION'/SYN
3	#1 AND #2
4	#1 AND #2 AND [HUMANS]/LIM AND [EMBASE]/LIM